PB-220 479

PHYSICAL, CHEMICAL AND MICROBIOLOGICAL METHODS OF SOLID WASTE TESTING

NATIONAL ENVIRONMENTAL RESEARCH CENTER

PREPARED FOR
ENVIRONMENTAL PROTECTION AGENCY

MAY 1973

Distributed By:

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PB 220 479

EPA-6700-73-01

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U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL ENVIRONMENTAL RESEARCH CENTER, CINCINNATI
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212

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D.F. BENDER, M.L. PETERSON,
AND H. STIERLI, EDITORS

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL ENVIRONMENTAL RESEARCH CENTER, CINCINNATI
MAY 1973

REVIEW NOTICE

The Solid Waste Research Laboratory of the National Environmental Research Center, Cincinnati, U.S. Environmental Protection Agency, has reviewed this report and approved its publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This manual is drilled for use with a three-ring binder.

BIBLIOGRAPHIC DATA SHEET	1. Report No. EPA-6700-73-01	2.	3. Recipient's Accession No. PB-220 479

4. Title and Subtitle

Physical, Chemical and Microbiological
METHODS OF SOLID WASTE TESTING

5. Report Date
May 1973

6.

7. Author(s)
D. F. Bender, M. L. Peterson, and H. Stierli, eds.

8. Performing Organization Rept. No.

9. Performing Organization Name and Address

U.S. Environmental Protection Agency
Office of Research and Monitoring
National Environmental Research Center
Cincinnati, Ohio 45268

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Address

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstracts

This publication describes the physical, chemical, and microbiological methods used by the Solid Waste Research Laboratory to analyze solid wastes and solid waste related materials. The latter includes products and potential pollutants resulting from the handling, processing, disposal or recycling of solid wastes. It is intended to serve both as a laboratory manual for technicians and as a reference for analysts who must evaluate the data.

17. Key Words and Document Analysis. 17a. Descriptors

Manuals, Tests, *Chemical analysis, Bacteriological examinations, Waste disposal

17b. Identifiers/Open-Ended Terms

*Solid waste, Refuse disposal, Environmental engineering, Pollution, Waste effluents, Microbiological examination

17c. COSATI Field/Group

18. Availability Statement Release to the public	19. Security Class (This Report) UNCLASSIFIED	21. No. of Pages 212
	20. Security Class (This Page) UNCLASSIFIED	22. Price $6.75/$1.45

FORM NTIS-35 (REV. 3-72)

USCOMM-DC 14952-P72

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL ENVIRONMENTAL RESEARCH CENTER
CINCINNATI, OHIO 45268

Dear Friend of the Environment:

We are pleased to send you *Physical, Chemical, and Microbiological Methods of Solid Waste Testing*. This manual represents many man-years of studying, investigating, and testing to develop and adapt methods for analyses related to solid wastes. Several additional methods are being prepared for future publication. These supplemental methods will be distributed as they become available.

This manual is designed for the researcher, laboratory worker, or student, and the format permits the user to insert it into a three-ring binder and add his own working notes. It also makes possible replacing or adding methods as they are developed or redesigned.

If your organization needs additional copies, the Technical Information Office, National Environmental Research Center, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, can supply them on request. However, please address any comments or questions you may have directly to me.

Sincerely yours,

Robert L. Stenburg

Robert L. Stenburg, Director
Solid Waste Research Laboratory

FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment–air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

This publication of the National Environmental Research Center, Cincinnati, describes the physical, chemical, and microbiological methods used by the Solid Waste Research Laboratory to analyze solid wastes and solid waste related materials. The latter includes products and potential pollutants resulting from the handling, processing, disposal, or recycling of solid wastes. Five years of evaluating and applying available methods in incinerator testing studies, municipal waste characterization studies, and solid waste utilization schemes are represented. Although the results of these studies have been published and are referenced throughout, the detailed methodology is being made available in this single source for the first time.

A. W. Breidenbach, Ph. D.
Director
National Environmental Research Center,
Cincinnati

PREFACE

This publication is a compilation of methods used by the Solid Waste Research Laboratory of the National Environmental Research Center in Cincinnati, Office of Research and Monitoring, U. S. Environmental Protection Agency, to perform various physical, chemical, and microbiological analyses in the field of solid waste management. It is not intended to be a complete manual, but the first edition of a growing collection of methods used by the Solid Waste Research Laboratory to test refuse, incinerator residue, incinerator residue quench water, incinerator scrubber water, sanitary landfill leachate, and the products of laboratory and pilot studies directed toward reclaiming waste through physical, chemical, and biological transformation.

The preparation of this manual was under the general direction of Mr. Harry Stierli, Chief, Support Services Branch, Solid Waste Research Laboratory. Dr. Daniel F. Bender, Project Manager, Laboratory Support Project, Support Services Branch, edited Part I, Physical Methods, and Part II, Chemical Methods. Part III, Microbiological Methods, was prepared by Dr. Mirdza L. Peterson, Senior Research Microbiologist, during her assignment with the Support Services Branch.

Most methods previously reported in the literature have required some modification for application to the analysis of solid wastes. The procedures presented in this compilation were modified where necessary, evaluated, and applied by the various authors.

Robert L. Stenburg, Director
Solid Waste Research Laboratory

INTRODUCTION

Daniel F. Bender and Harry Stierli

Because of the many requests for the information contained in this publication, we decided to print this first edition in a form that is incomplete from the standpoint of our overall plan. Waiting for a state of completion would delay the release of the often requested information. The fact that we have already planned for a second edition containing much more information does not reduce the value of this edition. A great deal of the information that will eventually be added is available in the literature, merely awaiting compilation to produce it in a single source in the format we have chosen. Other information to be added will result from current research, primarily on samples of leachate from sanitary landfills.

In choosing the format for presenting these methods, we concentrated on specific laboratory directions with adequate step-by-step explanations and only enough discussion to provide a sound general background. The evaluations, which employ more sophisticated statistical approaches, add another dimension. Thus, we are attempting to create both a laboratory manual for the technician who must produce the information and a sound, though not in-depth, theoretical background for the analyst who must evaluate the information. In addition, some of the more sophisticated methods we have used (for example, those for analyzing reclamation products) will have added value for persons involved in research rather than in testing or monitoring.

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PART I
PHYSICAL METHODS

INTRODUCTION

Eventually this section will consist of (1) a practical, statistically based method of field sampling for refuse, (2) sample handling and transporting characteristics, and (3) a discussion of laboratory sample selection from the bulk field sample as well as the currently included method of sample preparation for solid samples. When a general sample preparation procedure has been developed for leachate samples, it will be included as well. One other physical method of importance involves the application of a classification system to refuse samples (paper, glass, metal, etc.). A review of the many schemes that have been developed is planned for future editions of this publication.

LABORATORY PROCEDURE FOR THE PREPARATION OF SOLID WASTE RELATED MATERIALS FOR ANALYSIS

Israel R. Cohen*

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*Research Chemist, Solid Waste Research Laboratory, National Environmental Research Center Cincinnati.

DISCUSSION

The term "solid waste related materials" as used here refers to (a) raw refuse that is delivered to an incinerator or landfill for disposal, (b) residues of the incineration process, and (c) refuse in various stages of composting. It is assumed that major glass, ceramics, and metal components have been removed before submission to the laboratory. The three basic operations required to prepare solid waste related materials for detailed analysis are drying, grinding or pulverizing, and mixing. The end products of these operations must be so thoroughly homogenized that portions weighing as little as 100 to 200 mg may be extracted for analysis with full confidence that they are uniform and representative. Thus, any lack of precision or accuracy in the final results cannot be ascribed to the sample.

APPARATUS

Basically the apparatus needed to perform the above operations include ovens (preferably mechanical convection), cutting, grinding, and pulverizing tools, balances, and a mixing device. The following is a list of apparatus that has been found useful:

1. Ovens, Freas model 114, Thelco model 28, Blue M model POM-326 FXX
2. Pans for drying
3. Balance, Ohaus heavy-duty solution balance
4. Balance, top loading, 1,000-g capacity
5. Desiccator cabinet, large capacity
6. Shears, heavy duty
7. Hatchet
8. Saw
9. Plastic sheet
10. Sample splitter, Gilson, large capacity
11. Hammermill, W-W Grinder, Model F-21-P
12. Wiley mill #3
13. Micromill, Weber Bros. laboratory pulverizing mill
14. Ore pulverizer, Iler
15. Sieves, 10 mesh and 60 mesh
16. Sieve shaker
17. Rotating mixer
18. Miscellaneous gloves, scoops, funnels, containers, brushes, etc.
19. Dust collector, Norblo, bag type size 48-3, type BA-2.3

SAFETY PRECAUTIONS

When handling refuse, the analyst should use gloves if possible (neoprene-coated canvas). He should also wear some sort of face mask, such as a surgical mask, when preparing samples, especially when they are in finely divided form. It is advisable to wear a transparent plastic face shield while feeding material into the hammermill. The analyst should not use his hand to help push material into the hammermill past the feed slot; a stick can be used if necessary. Do not open any grinding device while it is running. If a mill clogs, turn off the motor before clearing apparatus.

PROCEDURE FOR COMBUSTIBLES

The procedures for all types of organic materials are essentially similar, if it is taken into account that compost samples have already been coarsely ground either in a hammermill or rasping device. It is preferable to dry the material before grinding. If the sample is small (3-5 lb of moist compost, or less of a bulkier material) the drying can be carried out conveniently in a large laboratory oven. If the complete sample is dried, an industrial-size oven is most convenient. Where the latter is not available, a preliminary grinding step in a hammermill is usually necessary.

Drying

For a small sample, use a laboratory oven; for a large sample, use an industrial oven.

Procedure	Comments
1. Weigh pan or pans.	
2. Transfer material to pan and reweigh.	
3. Note weight of sample.	
4. Dry in oven at 70 to 75 C overnight or for 24 hr.	
5. Remove sample and allow to cool, preferably in desiccator.	5. If a desiccator is not available, allow sample to cool covered with aluminum foil.
6. Reweigh.	
7. Replace in oven for 1 to 2 hr.	
8. Repeat steps 5 and 6.	
9. If the weight loss is less than 1 percent of total previous weight loss, calculate percent of moisture from total weight lost.	
10. If weight loss is over 1 percent of total previous loss, dry for an additional hour. Continue intermittent drying until the change in calculated moisture is within the limit set in step 9.	10. Example: 0.10 percent in 10 percent moisture 0.40 percent in 40 percent moisture

Grinding in Hammermill

This procedure does not apply to compost or other previously ground material.

Dried sample.

Procedure	Comments
1. Place sample collection box under mill.	
2. Plug lead into power outlet.	
3. Open cut-off in duct of dust-collecting system.	
4. Start blower.	
5. Oil grinder bearings with engine oil.	5. Motor bearings are permanently lubricated.
6. Put on personal safety equipment.	
7. Start motor.	

8. Feed sample into mill.	8. If components of sample have been segregated by categories, mix these during feeding. Tear thick magazines into thinner sections and separate thick wads of paper. Remove all metal objects (staples in magazines and cartons, bottle caps, metal buttons or staples on jeans and work clothes) and all other noncombustibles (glass, ceramics, etc.) not previously removed from the sample. Place these in a container marked "Discard." Cut articles of clothing into smaller sections before feeding into mill and saw or chop large pieces of wood or thick branches into smaller pieces. Close metal inlet door after inserting each batch of material.
9. Turn off grinder motor.	9. Remove electrical plug from outlet.
10. Turn off blower.	
11. Clean out grinder and add material to ground sample.	11. Remove clean-out plate at rear of grinder. Remove threads wound around shaft between hammers and cut long strings or threads into short pieces. Use electric light to illuminate dark areas. Scrape off bits of material caught in crevices.
12. If the sample weighs appreciably more than 2 lb, reduce it to about this weight by mixing on a plastic sheet and quartering or passing it through a sample splitter. For this purpose, the large-size splitter should be used with apertures about 4 in. wide.	

Undried sample.

Procedure	Comments
1. - 11. Steps 1 through 11 for the undried sample are the same as steps 1 through 11 for the dried sample.	1. - 11. More care must be exercised in cleaning the inside of the hammermill, since moist material will stick to the walls of the mill more readily. It is advisable to line sample collection box with plastic film or bag.
12. Spread ground product on a sheet of plastic and mix rapidly without compacting.	12. The sample may be mixed by manipulating the corners and sides of the sheet to move the particles from one area to another. A spade is often useful in this operation. Compaction of the particles reduces their mobility.
13. Quarter the material down to manageable size.	13. About 3 lb is a convenient sample size; store remainder in a labeled, plastic bag.
14. Dry sample as outlined under "Drying".	

Compost.

A compost sample, as received in the laboratory, may appear to be quite wet or dry. In either case, the sample should be dried before proceeding any further (unless assurances are received that the sample has been dried). Drying is carried out as detailed in that section.

Compost usually contains varying quantities of glass and ferrous and non-ferrous metal. If the sample has been relatively finely ground, it is not practical to remove the glass or non-ferrous metal. The ferrous metal can be picked out by combing the sample with a strong magnet. If the sample is quite coarse, much of the glass will be of a size that can be removed without too much difficulty by judicious sieving and by the use of a current of air to scatter the lighter organic particles. If the carbon/nitrogen ratio is to be determined, identifiable plastic should be removed because it is not degradable. Removal of glass and metal reduces the wear on the Wiley mill knives. After following these preliminary procedures, continue with those in "Fine Grinding."

Fine Grinding

The material that has been reduced in a hammermill and dried is now to be ground in a Wiley mill so that it will at least pass through a 2-mm sieve and preferably through a 1-mm sieve.

Procedure	Comments
1. Put a 2-mm sieve into a Wiley mill and close mill.	
2. Open cut-off in dust collection duct.	
3. Position container under delivery spout.	3. A standard Mason jar can be screwed directly into the spout. If the ground sample is caught in a different type of container, it is advisable to provide one with a covering that has a hole through which the spout may be inserted.
4. Replace the 2-mm sieve with a 1-mm sieve and regrind the sample.	4. The finer the grind, the more uniform the sample can be made.
5. Brush out all inside surfaces of the mill into a separate container.	5. This procedure includes the hopper, the walls, the screen, and the spout. Remove as much as possible of the material wedged between the knives and their housing.
6. Put this material through a micro mill.	6. On the Weber mill, the 0.05-in. or 0.10-in. screen may be used. For small samples, substitute a long, narrow plastic bag for the canvas bag provided.
7. Add the product to the main sample and mix.	

Mixing

The final mixing or homogenization is accomplished by transferring the sample to a suitable container that will be no more than half-filled by it. The container is closed tightly, positioned in the rotating mixer, and allowed to mix for not less than 1 hr, and preferably for 2 hr. The mixed sample may then be reduced in size, if desired, by passing it through a sample splitter or by quartering.

The Discard

Weigh all metal, ceramics, plastic, and glass removed during processing. This information will be used later in calculations.

PROCEDURE FOR NON-COMBUSTIBLES

Incinerator residues are usually wet when received and are dried at 100 to 105 C. The temperature or time of drying is not critical. A 10-lb sample or smaller is usually enough to dry.

Procedure	Comments
1. Weigh the dried sample.	1. If the moisture content is to be determined, this value is, of course, obtained in the routine procedure.
2. Work the sample over with a strong magnet to remove ferrous metal and magnetic iron oxide.	2. If the magnet is enclosed in a piece of cloth, it will be easy to remove the magnetic material clinging to it by separating the cloth from the magnet. The removal is otherwise quite difficult to effect.
3. Sieve the sample through a 1/4-inch mesh sieve and pick out glass and ceramics.	3. Though the pulverizer will grind this material, the removal of ingredients not contributing to the Btu value will enrich a sample whose Btu value is low under the best conditions.
4. Check the oil level in the pulverizer. The oil cup should be quite full.	4. The gears run in a bath of regular engine oil. Be careful not to get any oil into the sample compartment.
5. Adjust the movable pulverizer plate to give a maximum size of about 10 mesh.	5. This is accomplished by rotating the wheel with the holes in the rim (just ahead of the fly wheel) clockwise to give a coarse product and counter-clockwise to give a fine product.
6. Put the sample through the pulverizer.	6. When restarting the motor after emptying the product pan, it is advisable to empty the grinding chamber. Starting the motor against a load may cause burnout.
7. Screen the ground material through 10-mesh and 60-mesh sieves. The material that has been ground to pass through the 60 mesh sieve is the final sample. The remainder is reground with the plates placed together with minimum clearance.	7. It may be necessary to make multiple passes through the pulverizer to grind the whole sample through the 60-mesh sieve. The coarse material will often disclose bits of metal that have been burnished during the first passage through the pulverizer. These may be picked out and added to the discard (see steps 2 and 3).
8. Mix as indicated under "Mixing."	

PART II
CHEMICAL METHODS

INTRODUCTION

These methods have been modified, developed, evaluated, and/or applied to samples of material from solid waste handling, processing, reclaiming, and disposal methods. They were selected from many available methods used for water pollution, fuel, and fertilizer analyses, as referenced in each manuscript.

These are the methods that worked best for us. In the absence of a thorough study, we do not label these as recommended. Collaborative testing is necessary before such recommendations are possible.

The first consideration in method selection was that it give the necessary information for interpretation within a reasonable accuracy. We stressed simplicity, so that minimal training would be required, and speed, so that the samples could be analyzed before further decomposition could occur. Some research objectives involved more sophisticated methods, which are also included.

It is anticipated that in future editions this section will contain twice as many methods. Thorough evaluation and collaborative testing of all the methods will have to wait, however, until the urgency of finding methods that at least produce immediately needed information has subsided.

LABORATORY PROCEDURE FOR DETERMINING TOTAL HEAT OF COMBUSTION IN SOLID WASTES*

Donald L. Wilson†

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*This method is meant to be used in conjunction with the Parr Instrument Company's Technical Manual No. 130, Operating the Adiabatic Calorimeter.

†Research Chemist, Solid Waste Research Laboratory, National Environmental Research Center, Cincinnati.

DISCUSSION

The heats evolved in the complete combustion of many compounds in oxygen have been carefully determined. The method ordinarily used is to burn the substance in a combustion bomb and to measure the heat evolved.

The term "heat of combustion" refers to the amount of heat liberated per unit of substance burned. This process involves the change in enthalpy or heat content (H) of the system. The heat of combustion or ΔH_C described in this method is expressed in calories per gram of sample or British thermal units (Btu) per pound of sample.

The heat contents of various solid waste materials are important to some of the volume reduction processes used to dispose of waste. In the incineration process, for example, the operating efficiency of an incinerator can be measured by energy balance techniques, and analyzing heat contents of the solid wastes before and after incineration is essential. Knowledge of the heat value of solid waste is also necessary for incinerator design. In addition, heat content analyses need to be performed on incinerator residue and compost used for landfill since the stability of these waste products is a function of their heat contents.

To determine directly the heat change involved in a reaction, calorimeters are employed. A calorimeter consists essentially of an insulated container of water in which the reaction chamber is immersed. In an exothermic reaction, the heat generated is transferred to the water, and the consequent temperature rise of the water is read from an accurate thermometer immersed in it. The amount of heat evolved in the reaction may be calculated with data on the quantity of water present, its specific heat, and the change in temperature. Special corrections must be applied for radiation, rate of cooling of the calorimeter, temperature rise of the vessels, stirrer, etc. To compensate for these corrections, the heat capacity of the calorimeter is determined by burning a definite amount of a standard.

This method describes the capabilities and limitations of a Parr adiabatic calorimeter and furnishes the instructions needed to obtain best results with this apparatus. Using this method, the heats of formation of nitric acid and sulfuric acid are involved in the total heats of reaction; therefore, they are determined and the data are adjusted.

APPARATUS

Requirements

1. Balance, analytical, 150-g capacity, 0.1-mg readability
2. Beakers, one 30-ml, one 100-ml, and one 250-ml
3. Bottle, aspirator, plastic, 1-gal capacity
4. Bottle, carboy, 5-gal capacity
5. Bottle, reagent, 1-liter capacity
6. Bottle wash, plastic, 125-ml capacity
7. Bulb, rubber, 30-ml capacity
8. Buret clamps
9. Buret, 50-ml capacity, three-way stopcock
10. Calorimeter, adiabatic, Parr No. 1221, with Technical Manual No. 130

11. Calorimeter bomb, double valve, Parr No. 1101 (Fisher #4-392-1)
12. Calorimeter fuel capsule, stainless steel, Parr No. 43AS, (Fisher #4-385-5); eight or more
13. Calorimeter ignition unit, transformer type, Parr Series 2900
14. Calorimeter mercurial thermometers, Parr No. 1601; two
15. Calorimeter oxygen filling connection, safety type, Parr Series 1823
16. Calorimeter thermometer reading lens, Parr No. 3003; two
17. Calorimeter water heater, automatic, electric Parr Series 1500
18. Carver laboratory press, Model C or equivalent (Will #22562)
19. Carver test cylinder outfit or dies, specially made (internal diameter of cylinder is 3/4 in. and length of internal cylinder chamber is 3-1/4 in.;* similar to outfit with 1-1/8-in. internal diameter of cylinder (Will #22591)
20. Clamp, pinchcock
21. Desiccator
22. Weighing dish with lid, eight or more
23. Drying oven
24. Flasks, volumetric, one 2-liter and one 1-liter
25. Funnels, filling, one 80-mm diameter and one 250-mm diameter
26. Jack, laboratory
27. Pipet, volumetric, 1-ml capacity
28. Ring, support, with clamp, 4-in. outside diameter (O. D.)
29. Scissors
30. Stopwatch
31. Support stand, rectangular base, 24-in. rod; two
32. Tubing, glass, 1/2-in. inside diameter (I. D.), about 3 ft
33. Tubing, plastic, 1/4-in. I.D.; about 3 ft
34. Tubing, plastic, 1/2-in. I.D.; about 6 ft

Assembling

The general arrangement of the calorimeter and accessories may be found in the Parr Company's Technical Manual No. 130, pages 17 to 21. The procedure section contains instructions on when to plug in the water-heater cord.

For convenience, the distilled water is stored in a 5-gal carboy bottle with glass tubing extending through the lid to near the bottom. A plastic tube is connected to the glass tubing and a pinchcock clamp is used to prevent the flow of water. The sodium carbonate solution is stored in a 1-gal plastic bottle with aspirator. Plastic tubing of 1/4-in. I. D., connects the aspirator to the automatic filling buret. The plastic bottle rests on a laboratory jack atop a shelf, thus allowing the level of reagent to be higher than the solution within the buret. A 250-ml beaker is put beneath the buret to prevent spilling. A support ring is connected to a support stand about 1 ft above the base. The 1-ml pipet stored in an inverted filling funnel, 80 mm in diameter. For ease of pipeting, some distilled water is stored in a 30-ml beaker.

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*Although the smaller die is preferred, a larger die may be used with modifications in the procedure. The pellets are made thicker and broken into four or more parts by using a sharp object or breaking by hand. Handle the pellets as little as possible.

REAGENTS

Chemical Requirements

1. Benzoic acid, primary standard with calorific value known (Fisher #A-68)
2. Methyl orange, ACS grade (Fisher #M-216)
3. Oxygen, 99.5 percent pure (the oxygen should be prepared from liquid air, since oxygen prepared by electrolysis contains traces of hydrogen)
4. Sodium carbonate, Na_2CO_3, ACS grade, anhydrous
5. Steel wool
6. Wire, nickel-iron chromium alloy, No. 34 B. & S. gauge, wound on a card 10 cm long (Parr 45 C10)

Preparation

1. Sodium carbonate solution, 0.0725N: Dissolve 3.8421 g of Na_2CO_3 in distilled water and dilute to 1 liter (store in plastic bottle with aspirator).
2. Saturated methyl orange solution: Dissolve 0.5 g of methyl orange in distilled water and dilute to 1 liter (store in reagent bottle).
3. Wash solution: Pipet 1 ml of saturated methyl orange solution into a 1-liter flask and dilute to the liter mark with distilled water.
4. Ignition wire: Using 10-cm marks on card provided with wire, measure and cut off 10 cm of wire with scissors. Several 10-cm lengths may be made at one time and stored in a 100-ml beaker.

SAFETY PRECAUTIONS

See the Parr Company's Technical Manual No. 130, page 9, "Hazards of Operation."

SAMPLE PREPARATION

Total sample preparation procedures such as the drying and grinding techniques are described in detail elsewhere. In general, since this method restricts the quantity of sample analyzed to a weight of about 1.0 g or less that has been thoroughly dried, the entire sample (except glass, metals, and ceramics) must be ground until the particle size is reduced to less than 2 mm, dried until there is no more loss in weight, and then completely mixed before being analyzed.

Fluffy materials (standard benzoic acid, raw refuse, and compost) must be made into pellets in order to fit the metal capsules. The procedure for pelleting is:

Procedure	Comments
1. Using Carver laboratory press with 3/4-in. diameter punch and die, loosely fill the chamber 1/2 to 3/4 full of sample.	1. A 1-1/8-in. diameter punch and die may be used, but the pellet will have to be broken. If at all possible, the 3/4-in. punch and die should be used.

2. Compress the sample until the pressure is 5,000 to 7,000 lb, as read by the dial.	
3. Transfer the pellet to a weighing dish with lid.	3. Touch pellets as little as possible with hands.
4. Repeat for each determination.	4. See "Method Evaluation."

PROCEDURE

The following procedure applies to all solid waste materials and the benzoic acid standard.

To ensure precision and to keep from exceeding the capacity of the instrument, it is advisable that restrictions in Table 1 be followed.

TABLE 1
SAMPLE RESTRICTIONS

Sample type	Sample weight (g)	Specifics of analysis
Benzoic acid	1.0 ± 0.2	pelleted
Raw refuse	0.4 to 0.8	pelleted
Residue (combustible)*	0.4 to 0.8	pelleted
Residue (fines)†	0.8 to 1.2	combustion aid added
Fly ash	0.8 to 1.2	combustion aid added
Compost	0.4 to 0.8	pelleted

*Mostly readily combustible materials which remained on a 1/2-in. sieve during manual sorting at the incinerator site.

†Material remaining after combustibles, metal, glass, and ceramics removed from incinerator residue.

Procedure	Comments
1. Weigh sample into tared capsule. If the sample is in pellet form, it must be thin enough to keep the weight within restrictions. If a combustion aid is added, sandwich the sample evenly between pellets of the combustion aid, keeping the total weight of the combustion aid in excess of the sample weight.	1. a) Capsules should have been cleaned previously with steel wool and water, then dried and stored in desiccator until weighed. b) Use analytical balance and record to the fourth decimal place. c) Duplicate or triplicate samples are needed ("Method Evaluation"). d) Combustion aid is pelleted benzoic acid. e) Remember: not more than 10,000 calories should be liberated in any one test. f) If the combustion aid is made with a die

	larger than the recommended size, the pellet must be broken to meet restrictions. Keep the larger pellet under all of the sample.
2. Bind the fuse wire to 4A and 5A electrodes.	2. See Parr Manual No. 130, page 28.
3. Place the capsule with sample in 5A electrode.	3. The fuse wire should be bent so that a loop is just ABOVE the sample.
4. Pipet one ml of distilled water into the No. 1101 bomb.	4. Use a rubber bulb for the pipeting. (Distilled water is stored in a 30-ml beaker.)
5. Place bomb head into the cylinder.	5. a) Make certain sealing ring is in good condition. b) ALWAYS keep cylinder in upright position and do not disturb the sample.
6. Screw cap down firmly by hand.	6. a) Keep outlet needle valve open. b) Parr Manual No. 130, Figure 4, page 11, may be helpful.
7. Close outlet needle valve.	
8. Remove inlet valve thumb nut.	
9. Attach filling tube with union nut firmly, by hand.	9. Use Parr, Series 1823. Consult the Parr Manual No. 130, pages 20 and 21.
10. Open the filling connection control valve SLOWLY.	10. a) Oxygen tank valve must be open. b) If the filling connection control valve is opened quickly, sample may come out of capsule, thus causing incomplete combustion.
11. Allow pressure to rise slowly until gauge reads 30 atmospheres.	11. Although the pressure range is 25 to 35 atm., according to Parr Manual No. 130, the same pressure must be used for the entire test.
12. Close connection control valve.	
13. Push sideways on ball knob under relief valve.	13. This step relieves gas pressure in connecting tube.
14. Detach connnecting tube.	
15. Replace thumb nut.	
16. Place bomb with its feet spanning the boss in the bottom of the bucket.	
17. Attach the thrust terminal to the bomb.	17. Lead wire should not extend above the bucket.
18. Lower bucket into the jacket with stirrer at rear.	18. Lower handle to back of bucket.
19. Place 2,000 ml (about 2,000 g) of distilled water (at room temperature) into the bucket.	19. a) Keep the amount of water constant throughout all tests, once standardization is completed. b) A 2-liter volumetric flask should be used.
20. Close the calorimeter by swinging the cover to the right and lowering it with the cam lever.	20. Parr Manual No. 130, figures 8 and 9, page 13, may be helpful.

21. Make SURE the pump and stirrer drive shafts are seated properly by seeing to it that the pump and stirrer pulleys are down as far as possible and move freely.
22. Lower the thermometers into the calorimeter.
23. With all valves before and after the water heater open, turn on the water slightly. Note: The two valves nearest the calorimeter are always kept completely open. NEVER close all four valves completely.
24. Wait until water runs from the discharge tube, then close the hot water valve and plug in the water heater.
25. Start the stir motor and, again, wait until the water runs from the discharge tube. (Occasionally the pulleys need to be tapped down to maintain circulation of the water.)
26. Adjust the cold and hot control valves so that the jacket temperature, "left" thermometer, is the same or slightly lower than the bucket temperature, "right" thermometer.
27. If there is no change in the bucket temperature after 1 or 2 min, record this temperature.
28. Press button on ignition unit and stopwatch at the same time.
29. During the rapid rise in bucket temperature (usually 5 min), keep the jacket temperature about the same or slightly lower than the bucket temperature.
30. When the system is approaching final equilibrium temperature, keep the jacket temperature within 0.1 degree of the bucket temperature.

21. If the water in the bucket is not being stirred, its temperature will decrease soon after the final increase because of the sample combustion.
22. The thermometers should be submerged about the same distance as during standardization.
23. a) If the water is turned on too much, leaks may occur.
    b) See Parr Manual No. 130, pages 18 and 19.
    c) Counter-clockwise turns open all valves.
24. CAUTION: The water flow and heating of the water continues throughout the use of the instrument; however, the water heater may become overheated if its electric cord is not occasionally disconnected for a few minutes.
26. a) The main water valve may need adjusting. Lowering the flow rate increases the control over water temperature.
    b) Read thermometers to nearest 0.005 F.
    c) See Parr Manual No. 130, pages 14 and 15.
    d) Occasionally check the thermometers for mercury separations and reunite the mercury, if necessary, in accordance with the thermometers' certificates.
27. Use reading lenses and record to the nearest 0.005 of a degree F.
28. Keep switch closed for 4 to 5 sec only.
29. The rapid rise in bucket temperature begins about 20 sec after button is pressed.

31. Record the final maximum bucket temperature.	31. a) The stable maximum temperature occurs when the same temperature is observed in three successive readings. b) Use reading lenses and record to the nearest 0.005 of a degree F.
32. With the cold water valve slightly open, close the hot water valve.	32. This procedure will allow the jacket water to cool to a starting temperature for the next analysis.
33. Stop the stir motor and wait for water to drain out of the cover.	33. Water has drained from the cover when the flow from the discharge tube returns to normal.
34. Raise the thermometer support with the thermometers to its top position.	34. The thermometer support should remain at all times while the cover is off.
35. Using cam lever, lift the cover and swing to the left.	
36. Lift the bucket out of the jacket, disconnect the thrust terminal wire, and remove the bomb.	
37. With a filling funnel, pour the water from the bucket into the 2-liter flask.	37. A 250-mm diameter funnel is used.
38. With a sponge, paper towel, or napkin, remove excess water from top of bomb.	
39. Slowly relieve the pressure from the bomb by opening outlet needle valve.	39. This step should require about 1 to 3 min.
40. Remove screw cap and bomb head.	40. Discard test if there is definite evidence of incomplete combustion.
41. Using a wash bottle with prepared solution, wash the head and all interior surfaces of the bomb; then collect the washings in a 250-ml beaker. Carefully lay the bomb head aside.	41. The bomb head and all interior surfaces should be rinsed with distilled water and allowed to drain before the next analysis.
42. Titrate the washings to a reagent color end point, using 0.0725 N sodium carbonate solution and record the milliliters of titrant.	42. a) Record the milliliters of titrant as calories, to the nearest tenth, involved in the heat of formation of nitric acid. b) Most often this value represents less than 5 percent of the final answer. If it represents more, however, save the solution for determining sulfur content (see the Parr manual No. 130, pages 37, 48, and 49).
43. Carefully remove all unburned pieces of fuse wire from the bomb head and measure their combined lengths with the card originally containing the wire.	
44. Record the calorific value (to the nearest tenth) of the wire used.	
45. Repeats steps 1 through 24 for each analysis.	45. Steps 23 and 24 are not repeated in a continuous operation.

46. At the end of each continuous operating cycle, disconnect the electric plug of the water heater and then turn off the main source of water.

STANDARDIZATION

The term "standardization" denotes the determination of the energy equivalent or water equivalent factor (W) of the system (see Parr Manual No. 130, page 23). Benzoic acid, primary standard with calorific value known, is the material used to determine the W factor. The benzoic acid is analyzed in the manner previously described under "Procedure."

CALCULATIONS

Standards

Compute the energy equivalent by substituting in the following equation:

$$W = \frac{\Delta H_C M + e_1 + e_3}{t}$$

where:

W = energy equivalent of calorimeter in calories per degree F
ΔH_C = heat of combustion of standard benzoic acid in calories per gram
M = mass of standard benzoic acid sample in grams
t = corrected temperature rise* in degrees F
e_1 = correction for heat of formation of nitric acid, in calories
e_3 = correction for heat of combustion of firing wire, in calories

Samples

Compute the calorific value per gram of sample by substituting in the following equation:

$$\Delta H_C = \frac{tW - e_1 - e_2 - e_3 - e_4}{M}$$

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*Temperatures are corrected for thermometer variations by using graphs furnished by the Parr Company for each thermometer. If the sample has a heat content above 100 or 200 calories per gram, however, this correction will have little effect on the final answer.

where:

ΔH_c = heat of combustion of sample in calories per gram*
t = corrected temperature rise in degrees F
W = average energy equivalent of calorimeter found by standardization, in calories per degree F
e_1 = correction for heat of formation of nitric acid, in calories
e_2 = correction† for heat of formation of sulfuric acid, in calories
e_3 = correction for heat of combustion of firing wire, in calories
e_4 = heat of combustion of combustion aid, in calories
M = mass of sample in grams; it should *not* include mass of combustion aid

METHOD EVALUATION

The accuracy of this method was established by analyzing sucrose, NBS #17. The average of three determinations was 3,981 calories per gram, which is 46 calories per gram or 1.2 percent from the true value.

This method can analyze solid waste materials to within 40 Btu per pound, depending on the type of sample,‡ therefore, this method should not be employed to analyze samples that contain less heat content than the pooled standard deviation for that type of sample (Table 2). To ensure precision, the particle size of the samples must be less than 2 mm or must pass through a 60-mesh sieve. The sample must then be thoroughly mixed before being analyzed.

The standard deviation of the water equivalent factor (w) was 10 calories per degree F for one analyst in 1968 and 6 calories per degree F for another analyst in 1970.

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*(cal/g) (1.8) = Btu/lb

†Correction is not necessary if considered in e_1 and if e_1 is not more than 5 percent of the final answer.

‡Types of solid waste in this method refer to only solid samples (domestic origin) such as raw refuse, incinerator fly ash, incinerator residue, and compost.

TABLE 2
STANDARD DEVIATION* OF THE Btu-PER-POUND DETERMINATION

Type of sample	No. of samples	Btu/lb Duplicates	Btu/lb Triplicates	Range (Btu/lb)
Raw refuse	10	57†	40	7,366 to 9,999
Residue:				
Fines‡	7	76	53	249 to 2,470
Combustibles§	7	76	54	4,457 to 8,182
Fly ash	7	56	40	Zero to 601
Compost:				
Without sewage sludge	2	––	173	5,661 to 6,118
With 6% sewage sludge	8	––	227	4,504 to 7,510
With 10-15% sewage sludge	2	––	110	3,915 to 4,174

*A variance estimate can be calculated from the duplicate (or triplicate) set of observations for each sample. The pooled variance is essentially an average of all such estimates for samples of a given type. It is assumed that a single underlying variance exists for all samples of a given type. The pooled variance is then the best estimate of this underlying variance. The pooled standard deviation is the square root of the pooled variance and is used to estimate the underlying standard deviation.

†The absolute value of the difference between duplicated observations should not exceed 1.96 (2) (s), confidence interval, or 158 Btu/lb, more than 5% of the time. The covariance between the duplicated observations was ignored.

‡Fines are materials remaining after most of the readily combustible substances have been removed by manual sorting. The sorting was performed at the incinerator sites and 1/2-in. sieve was employed to assist in the separation.

§Combustibles or organics are mostly the readily combustible materials. Unlike the fines, these materials are usually retained on a 1/2-in. sieve.

ACKNOWLEDGMENTS

The author gratefully acknowledges the assistance of Richard Carnes, Annella Johnson, Nancy Ulmer, Donna Barnet, Israel Cohen, and James Doerger in developing this method.

The author also wishes to thank the Division of Technical Operations, Office of Solid Waste Management Programs, for providing samples from incinerators, and the PHS-TVA Compost Plant, Johnson City, Tennessee, for supplying compost samples.

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LABORATORY PROCEDURE FOR DETERMINING POTENTIAL HEAT IN SOLID WASTES*

Donald L. Wilson†

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*This method is meant to be used in conjunction with "Laboratory Procedure for Determining Total Heat of Combustion in Solid Wastes."

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DISCUSSION

Not all solid waste samples with similar total heat contents (enthaplies, or heat of combustion values) are similarly ignitable or combustible. The readily available heat content of a solid waste sample, or its potential heat, could be an important criteria for evaluating the efficiency of an incinerator or for measuring the usefulness of incinerator residue.

Potential heat is not a new concept. The construction industries have long been interested in the potential release of heat of materials during fires (1). In the construction industries, potential heat is defined as "the difference between the heat of combustion of a representative sample of the material and the heat of combustion of any residue remaining after exposure to a simulated standard fire, using combustion calorimetric techniques." Conditions for a standard fire cannot be simulated when dealing with incinerators. However, since a combustion aid is employed in the method for total heat of combustion values (2), the ease with which solid waste materials ignite and burn to completion can be measured by applying the same calorimetric techniques except omitting the combustion aid and allowing only the flash heat of the ignition wire to ignite the sample.

The method presented here describes how to modify the total heat of combustion procedure to obtain the potential heat of combustion values in incinerator residue and fly ash samples. Other types* of solid waste materials usually do not have a potential heat value. Some incinerator residue and fly ash samples have negative potential heat values. Such samples are high in carbonates, which absorb heat upon decomposing (endothermic reaction).

With the total and potential heat values, the analyst can obtain the residual heat content of the sample. Residual heat content is defined as the total heat content minus the potential heat content. Residual heat value represents the heat content of a residue or fly ash sample that is not easily obtainable and would probably exist regardless of incinerator efficiency.

APPARATUS

The apparatus for this method is the same as that described in Reference 2.

REAGENTS

The chemical requirements and the preparation of reagents for this method are also the same as those described in Reference 2. Although benzoic acid is not used in this method as a combustion aid, it is required in standardizing the calorimeter.

SAFETY PRECAUTIONS

See Parr Technical Manual No. 130, page 9, "Hazards of Operation" (3).

SAMPLE PREPARATION

The techniques of sample preparation are the same as those outlined in Reference 2.

PROCEDURE

This procedure applies to all solid waste materials that normally require a combustion aid to determine total heat content. Since the mechanics are about the same as those outlined in Reference

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*The types of solid waste used in this method include only solid samples (domestic origin) such as raw refuse, incinerator fly ash, incinerator residue, and compost.

2 (except that there is no addition of a combustion aid), the details of the procedure are not repeated here. Note, however, that duplicate results may not agree if the sample partially ignites one time and not the next. This disparity very seldom occurs; but if it does happen, the analysis should be repeated until the sample ignites again. Failure to ignite can be detected by observing that the temperature rise is very slight and the answer is around zero. Poor positioning of the ignition wire can cause a sample to fail to ignite. The ignition wire must be installed, as instructed, close to the sample but not touching the sides of the sample container or the sample itself. The same restrictions on sample portions apply for both methods. Since a combustion aid is not used in this method, the sample is spread evenly on the bottom of the sample container.

STANDARDIZATION

The term "standardization" denotes the determination of the energy equivalent or water equivalent factor (W) of the system (see Parr Manual No. 130, page 23). Benzoic acid, primary standard with calorific value known, is the material used to determine the W factor. The benzoic acid is analyzed in the manner described in the section on "Procedure" in Reference 2.

CALCULATIONS

Total Heat Content

The formula for computing the total heat content of a sample is described under "Calculations" in Reference 2.

Potential Heat Content

The formula for computing potential heat content of a sample is the same as the formula for total heat content, except that the "e_4" term, which is the heat content of the combustion aid, is omitted.

Residual Heat Content

Compute the residual heat content of a sample by substituting in the following equation:

$$\Delta H_{c(R)} = \Delta H_{c(t)} - \Delta H_{c(P)}$$

where:

$\Delta H_{c(R)}$ = Residual heat of combustion of sample in calories per gram or in Btu per pound*

$\Delta H_{c(t)}$ = Total heat of combustion of sample as determined by the total heat of combustion method used in conjunction with this method

$\Delta H_{c(P)}$ = Potential heat of combustion of sample as determined by the method described here

METHOD EVALUATION

This method can analyze solid waste materials with low or even negative potential heat contents. Duplicate observations of the same sample will agree 95 percent of the time within about 25.8 to 144 Btu per pound, depending on the type of sample and whether or not some sample combustion takes place (Table 1). Although a temperature rise was always observed in these tests, a lowering of initial temperature is very possible. For example, a sample high in carbonates, which decompose easily, can produce a negative temperature change when the carbonates decompose upon being heated by the ignition wire. To ensure precision, samples should be prepared in the manner described in Reference 2.

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*Values must be expressed in the same units throughout the formula.

TABLE 1

POOLED STANDARD DEVIATION* OF THE POTENTIAL Btu-PER-POUND DETERMINATION

Type of sample	Number of samples	Duplicate observations (Potential Btu/lb)	Range (Btu/lb)
Residue (fines)†	9	52.0‡	597 to 2,580
Residue (fines)	8	13.8	-24.1 to 25.9
Fly ash	13	9.3	-35.3 to 12.3

*A variance estimate can be calculated from the duplicate (or triplicate) set of observations for each sample. The pooled variance is essentially an average of all such estimates for samples of a given type. It is assumed that a single underlying variance exists for all samples of a given type. The pooled variance is then the best estimate of this underlying variance. The pooled standard deviation is the square root of the pooled variance and is used to estimate the underlying standard deviation.

†Fines are materials remaining after most of the readily combustible substances have been removed by manual sorting. This material passed through a ½-inch sieve.

‡The absolute value of the difference between duplicated observations should not exceed $1.96 (\sqrt{2})(s)$, confidence interval, or 144 Btu per pound, more than 5 percent of the time. The covariance between the duplicated observations was ignored.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to J. U. Doerger for performing the laboratory analyses necessary to calculate the precision of this method. The author also thanks Betty Grupenhoff of the Office of Solid Waste Management Programs for special computer assistance.

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LABORATORY PROCEDURE FOR DETERMINING PERCENT ASH AND PERCENT WEIGHT LOSS OF SOLID WASTES ON HEATING AT 600 C

Nancy S. Ulmer*

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DISCUSSION

Incineration has been employed since 1874 as a method of solid waste reduction(1). Although early incinerator designs reflected the desire for recovering released heat for steam production and for eliminating or reducing potentially hazardous materials, recent designs have been influenced by a need for more efficient solid waste reduction and lower costs. Engineers and scientists have used various parameters* (for example, the percent heat released, and the percent reduction in volume, weight, and volatile solids) to evaluate the reduction efficiency of incinerator designs and to plan for the economic disposal of the residue (2, 3).

A variety of analytical procedures have been employed for the determination of the volatile solids in solid wastes. Researchers such as Kaiser(4) have adapted the ASTM standard procedure for coal(5) to the analysis of refuse and residue. The heating of the sample in a closed crucible, as directed in the ASTM technique, prevents the oxidation of elemental carbon, however, and results in a weight loss attributable only to the volatilization of hydrocarbons. Other investigators, such as Schoenberger (6) and Wiley and Spillane(7), have attained more complete oxidations by utilizing open-crucible techniques.

A modification of the procedure proposed by the American Public Works Association(8) has been used extensively by the research services laboratory staff for the characterization of refuse, residue, and fly ash. Briefly, the technique involves (a) the introduction of two 2-g samples (each contained in a porcelain crucible) into a cold muffle furnace, (b) the gradual increase in furnace temperature to 600 C, and (c) a 2-hr exposure of the samples to the latter temperature. While the crucibles are in the furnace, the lids are either removed or tilted at an angle sufficiently large to insure the circulation of air over the samples. After the 2-hr exposure to heat has been completed, the crucibles are removed from the furnace, immediately covered with their respective lids, and cooled to room temperature in a desiccator. The percent ash and the percent weight loss on heating at 600 C are calculated† after observing the weight lost by each sample.

A detailed description of the procedure is presented in the following sections. The details of a Solid Waste Research Laboratory independent study on the applicability of this method for the characterization of solid wastes is reported elsewhere(9).

EQUIPMENT

1. Balance, analytical, 0.1-mg readability
2. Boxes, sample storage, seamless aluminum, 3½-in. diameter, with tight-fitting lids (Fischer No. 3-485)
3. Crucibles, porcelain, Coors, high form, size 2, with lids
4. Desiccator, large, either Pyrex or stainless steel cabinet-type
5. Furnace, muffle, with indicating pyrometer and temperature controller (Lindberg Hevi-Duty Muffle Furnace, Model 51333; with automatic Control Console, Model 59545; platinum, platinum – 13 percent rhodium thermocouple; and six extra globar, silicon carbide heating elements)
6. Gloves, asbestos, 11 in. long, provided with separate places for thumb and four fingers

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*In this paper the term parameter means a variable or characteristic of interest.

†Since the observed decrease in sample weight reflects not only the weight lost by the vaporization of volatile materials and the combustion of fixed carbon, but also any weight gained (for example, by oxidation of metallic components during heating), the term "weight loss on heating" appears more appropriate than the term "volatile solids." The term "ash" denotes, of course, the material remaining in the crucible after heating the sample.

7. Gloves, cotton, unlined (No. 2200 MUH, Wash-Rite, Inc., 1410 Cornell, Indianapolis, Indiana)
8. Ink, ceramic, marking, Coors, black
9. Mats, board, asbestos, 1/8 by 12 by 12 in.
10. Oven, drying, forced-air (or mechanically convected), capable of maintaining temperatures up to 110 C
11. Pen, steel, for applying ceramic ink
12. Potentiometer, direct reading, with chromel-alumel thermocouple and scale, capable of reading temperatures from 0 to 1,000 C (West Pyrotest, Model 9B, West Instrument Corp., Schiller Park, Illinois)
13. Spatula (Scoopula, Fischer No. 14-357)
14. Tongs, crucible, nickel-plated, 20 in. long (Fischer No. 15-208)

REAGENTS

1. Inert standard (ground McDanel, high-temperature porcelain combustion tube fragments)
2. Combustible standard (A.C.S. sucrose or benzoic acid)

SAMPLE PREPARATION

A solid waste sample must undergo physical preparation before its characterization is initiated in the laboratory. First it must be dried to constant weight, preferably in a forced-air (or mechanically convected) oven. A temperature of 70 to 75 C should be used to dry municipal refuse (or compost); incinerator residue and fly ash may be dried at 100 to 105 C. The particle size of the dried sample should be reduced to 2 mm or less using a hammermill, pulverizer, or laboratory mill. To ensure sample homogeneity, the ground samples should be thoroughly mixed. Finally, since samples may absorb moisture during the grinding and mixing processes, they should be redried for 3 hr at the previously specified temperatures and then stored in a desiccator until the analyses are completed.

SAFETY PRECAUTIONS

The following suggestions apply to the analysis of samples:

1. Personal burns can be prevented by introducing the crucibles into a cool furnace and employing asbestos gloves and long tongs while handling the hot crucibles and lids.
2. Bench-top damage can be prevented by using asbestos mats to support the hot crucibles and lids before their introduction into the desiccator.
3. Breakage of desiccator glass can be prevented by (a) cooling the hot, covered crucibles a few minutes before inserting them into the desiccator and (b) avoiding any direct contact of warm crucibles with the glass.

PROCEDURE

The determination of the percent ash and the percent weight loss of each blank, standard, or solid waste sample on heating at 600 C should be performed in duplicate as follows:

Procedure	Comments
1. Transfer a numbered, clean, dry crucible and lid from the desiccator to the pan of an analytical balance.	1. (a) Using a steel pen, apply a Coors porcelain ink number to the exterior surface of each crucible and lid. Fire the dried ink either with a gas flame or by heating in a muffle

	furnace at 600 C for 1 hr. (The latter technique also ensures low blank values for new crucibles and lids.) (b) Clean each crucible and lid in warm detergent with a non-metallic brush; rinse first with tap water, then with distilled water; dry at 105 C for 1 to 2 hr; assemble and then cool in a desiccator until needed. (c) In steps 1 through 6, wear cotton gloves to avoid finger printing the crucible and lid.
2. Weigh the crucible and lid to the nearest 0.0001 g.	
3. Add approximately 2 g of the prepared sample.	3. Do not add sample to the "blank."
4. Weigh the crucible, lid, and sample to the nearest 0.0001 g.	
5. Transfer the covered crucible to a cool muffle furnace.	5. (a) Use a tray or stack of asbestos mats to transport a number of crucibles simultaneously. (b) WARNING: Introduction of samples into a hot furnace may result in their sudden ignition and loss, and in burns to the analyst. (c) Space the crucibles to permit air circulation around each. (Nine crucibles can be arranged in five rows in the Lindberg furnace.)
6. Carefully tilt each crucible lid at an angle sufficiently large to insure the circulation of air over the sample.	6. Some analysts remove the crucible lids from samples that do not sputter on ignition. Care must then be exercised to return each lid to the proper crucible after the heating is completed.
7. Gradually heat the muffle furnace to 600 C.	7. (a) If using the Lindberg furnace, dial in the desired setting on the digital set point after turning the power and control switches to the "on" position. (b) It has been observed that higher digital settings are required to achieve 600 C as the elements of the Lindberg furnace age. It is therefore recommended that the Lindberg furnace temperature be monitored once a week with an independent potentiometer and a chromel-alumel thermocouple. The latter may be introduced into the furnace through the small space surrounding the door. (c) A 30-minute period is required to heat the Lindberg furnace to 600 C.

8. Maintain the furnace temperature at 600 C for 2 hr.	
9. Then turn off the furnace and immediately transfer first a lid, then its corresponding crucible to a stack of at least three asbestos mats. Recover the crucible immediately.	9. (a) In steps 9 and 10, use an asbestos glove and pair of tongs while handling the hot crucibles. (b) Do not place the hot crucibles or lids directly on table tops or metal trays.
10. Allow the covered crucibles to cool for 3 to 5 min., then transfer to a desiccator.	10. Do not permit the hot crucibles to touch the glass of the desiccator.
11. After the crucible, lid, and sample have cooled to room temperature, weigh them to the nearest 0.0001 g.	11. (a) A hot crucible, lid, and sample usually cool to room temperature in 1 to 2 hr. (b) Wear a cotton glove to avoid fingerprinting the crucible and lid during their transfer to the balance.
12. Calculate the initial and final weights, the weight loss on heating, the percent ash, and the percent weight loss on heating of each sample.	12. (a) See the section on calculations. (b) The means of the two determinations of the percent ash and the percent weight loss on heating are usually reported.

STANDARDIZATION

Before initiating the characterization of solid waste samples, the analyst should evaluate his technique by determining the percent ash or percent weight loss on heating a combustible standard (for example, benzoic acid or sucrose), an inert standard (for example, ground McDanel high-temperature combustion tube fragments), and three mixtures (for example, 3:1, 1:1, and 1:3 parts by weight) of a combustible and an inert standard. (See the discussion of accuracy in Method Evaluation.)

Initially and periodically thereafter the analyst should also evaluate the applicability of his technique for cleaning and drying the crucibles and lids. The weight change on heating a properly cleaned and dried, but empty, covered crucible (or blank) should not exceed 0.0004 g.

CALCULATIONS

The initial and final sample weights, the weight loss on heating (WLOH), the percent ash, and the percent WLOH may be calculated as follows:

Initial sample weight (g) = B – A

Final sample weight (g) = C – A

Weight loss of the sample on heating (g) = B – C

$$\% \text{ ash} = \frac{100\,(C - A)}{(B - A)}$$

$$\% \text{ WLOH} = \frac{100\,(B - C)}{(B - A)} = 100 - \text{percent ash}$$

Where
A = the initial weight of the crucible and lid (g)
B = the initial weight of the crucible, lid, and sample (g)
C = the final weight of the crucible, lid, and sample (g)

METHOD EVALUATION

The mean observed percents WLOH of benzoic acid, sucrose, and mixtures of sucrose and McDanel combustion tube fragments were always ≥99.92 percent of the theoretical percent WLOH (Table 1). Although the mean observed percent WLOH of the 3:1 (parts by weight) mixture of sucrose and combustion tube fragments was 100.45 percent of the theoretical percent WLOH, no weight loss was ever observed when the combustion tube fragments were heated alone.

TABLE 1

ACCURACY OF THE METHOD

Type and identity of sample	Theoretical % WLOH (T)	Mean observed % WLOH (M)	(100 M/T)
Combustible standards:			
Benzoic acid	100	100.00	100.00
Sucrose	100	100.00	100.00
Sucrose-McDanel combustion tube, parts by weight:			
3:1	75	75.34	100.45
1:1	50	49.97	99.94
1:3	25	24.98	99.92
Inert standard:			
McDanel combustion tube	0	0.00	—

The reproducibility of this procedure may be determined by calculating the standard deviations of the determinations, the standard errors of the means of the determinations, and the coefficients of variation. A review of our observations and calculations (Table 2) indicates that the standard deviations of the determinations of the percents WLOH of the Delaware County, Pennsylvania, solid waste samples and the standard errors of the corresponding mean percents WLOH often exceeded and varied more than those of the standard samples; but the coefficients of variation were always less than 0.05. The nature or composition of the solid waste samples and the method of sample preparation may, of course, influence the reproducibility.

Although sufficiently accurate and precise determinations of the percent ash and the percent weight loss of solid wastes on heating at 600 C may be obtained with this procedure in 4 hr, the applicability of the two defined parameters may be limited. Research done by the Solid Waste Research Laboratory has recently demonstrated that some residue and fly ash samples contain significant quantities of carbonate(10). Since the percent WLOH of Na_2CO_3 and $CaCO_3$ on heating at 600 C is very low, even in the presence of benzoic acid(11), engineers and scientists may find it advisable to use percent WLOH data collected at 960 C to evaluate the reduction efficiency of some incinerators.

TABLE 2

REPRODUCIBILITY OF THE METHOD

Sample identity	RSL No.	Mean observed % WLOH (M)	Standard deviation of the deter-minations (S)	Standard error of the mean ($S/\sqrt{2}$)	Coefficient of variation (S/M)
Combustible standards:					
Benzoic acid		100	0.00	0.00	0.00
Sucrose		100	0.00	0.00	0.00
Sucrose-McDanel combustion tube, parts by weight:					
3:1		75.34	0.06	0.04	0.00
1:1		49.97	0.14	0.10	0.00
1:3		24.98	0.01	0.01	0.00
Inert standard:					
McDanel combustion tube		0.00	0.00	0.00	–
Solid wastes:*					
Refuse	99	87.58	0.08	0.06	0.00
	100	89.03	0.10	0.07	0.00
	101	86.08	0.06	0.04	0.00
	102	86.76	1.01	0.71	0.01
	103	91.38	0.37	0.26	0.00
Residue:					
Combustible fraction†	105	52.04	0.52	0.37	0.01
	107	56.48	0.90	0.64	0.02
	109	62.96	0.65	0.46	0.01
	111	49.30	0.22	0.16	0.00
	113	61.80	2.21	1.56	0.04
Fines fraction‡	104	15.85	0.17	0.12	0.01
	106	12.63	0.37	0.26	0.03
	108	12.92	0.06	0.04	0.00
	110	11.82	0.16	0.11	0.01
	112	25.63	0.47	0.33	0.02
	112a	49.78	0.25	0.18	0.01
Fly ash	90	1.80	0.02	0.01	0.01
	91	5.06	0.00	0.00	0.00
	92	4.51	0.04	0.03	0.01
	93	3.81	0.00	0.00	0.00
	94	1.78	0.03	0.03	0.02
	96	2.75	0.01	0.01	0.00
	97	4.58	0.02	0.01	0.00

***These samples were collected at Incinerator No. 3, Delaware County, Pennsylvania.**

†The combustible fraction includes most of the residue that can be visually identified as containing food, paper, plastics, rubber, leather, wood, textiles, or garden wastes.

‡The fines fraction includes all the unidentified and some of the identifiable residue particles that pass through a 0.5-in. wire mesh sieve.

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LABORATORY PROCEDURE FOR THE GRAVIMETRIC DETERMINATION OF CARBON AND HYDROGEN IN SOLID WASTES*

Donald L. Wilson†

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*A description of this method appears in "Method for Macrodetermination of Carbon and Hydrogen in Solid Wastes," D. L. Wilson, Environmental Science & Technology, 5:609-614, July 1971.

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DISCUSSION

Carbon and hydrogen are determined gravimetrically after combusting a weighed, dry, uniform sample in an atmosphere of oxygen with a closed system and after fixing the combustion products in an absorption train (Figure 1). The procedure (Figure 2) is designed to measure the total carbon and hydrogen in dry solid wastes samples.

The 38-in. (1 1/2-in. O. D.) combustion tube, starting at a point 16 1/4 in. from the non-tapered end contains the following materials in the order named (Figure 3): Special mixture of asbestos, platinized asbestos, and aluminum oxide; lead chromate; asbestos; copper dioxide; asbestos; lead chromate; silver wool; lead dioxide; and silver wire. The plain asbestos is employed as a support between the materials in the tube. The platinized asbestos located near the sample combustion area assists in the combustion of condensed ring systems, particularly those containing an angular methyl group that may evolve as methane and thus escape complete combustion. Aluminum oxide is used to absorb fluorine from the diffusing gaseous combustion products, and lead chromate oxidizes any SO_2 to SO_3 and, finally, to the nonvolatile sulfate ($PbSO_4$). Copper oxide converts any carbon monoxide to carbon dioxide, and lead dioxide retains the oxides of nitrogen as follows:

$$2NO + 2PbO_2 \xrightarrow{\Delta} Pb\,(NO_2)_2 \cdot PbO + 1/2\ O_2$$

$$2NO_2 + 2PbO_2 \xrightarrow{\Delta} Pb\,(NO_3)_2 + PbO + 1/2\ O_2$$

The sections of silver wire and silver wool, located near the tapered end of the combustion tube, absorb chlorine, bromine, and iodine from the gaseous combustion products before they diffuse from the combustion tube into the absorption tubes where magnesium perchlorate and Indicarb are employed to absorb the water vapor and carbon dioxide gas, respectively.

Samples containing (a) alkali or alkaline earths, as potash or calcium oxide, in the absence of sulfur or phosphorus or (b) phosphorus in the absence of alkali or alkaline earths can only be evaluated if an accelerator is added to the samples to ensure complete combustion and conversion of all the carbon to carbon dioxide. Iron chips (carbon free) are used as the accelerator. The iron ignites and starts the exothermic combustion reactions before the sample reaches the final induced temperature of 950 C.

This method is applicable to raw garbage, compost, incinerator residue, and other dry, general waste samples that can have a carbon content of 0.5 to 83.0 percent and a hydrogen content of 0.01 to 7.80 percent. Samples containing appreciable amounts of arsenic, antimony, bismuth, and mercury should not be analyzed unless further modifications in the procedure are made, since these elements would quickly deteriorate the combustion tube packing.

Since hydrogen is analyzed as water, it is essential that all of the moisture be removed from the samples before they are analyzed. All samples must be re-dried no more than a few days before the analyses are performed.

APPARATUS

Requirements

1. Asbestos boards, 6 x 6 x 1/8 in., and 12 x 12 x 1/8 in.
2. Baffle, oxygen (Sargent #S-21787)
3. Balance, analytical, 200-g capacity, 0.1-mg readability
4. Barge, combustion, high purity nickel (Fisher #7-647)

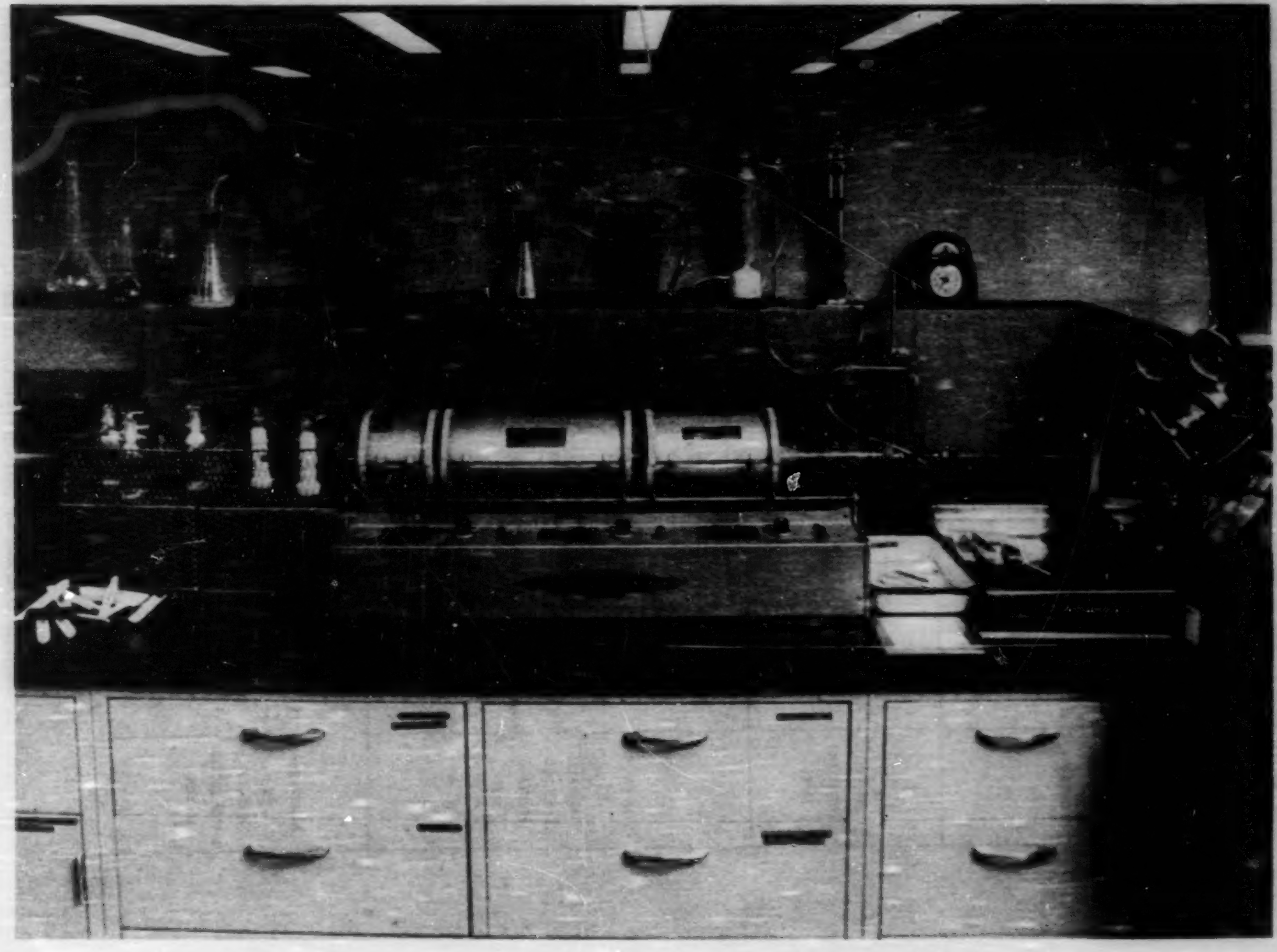

Figure 1. The carbon-hydrogen train.

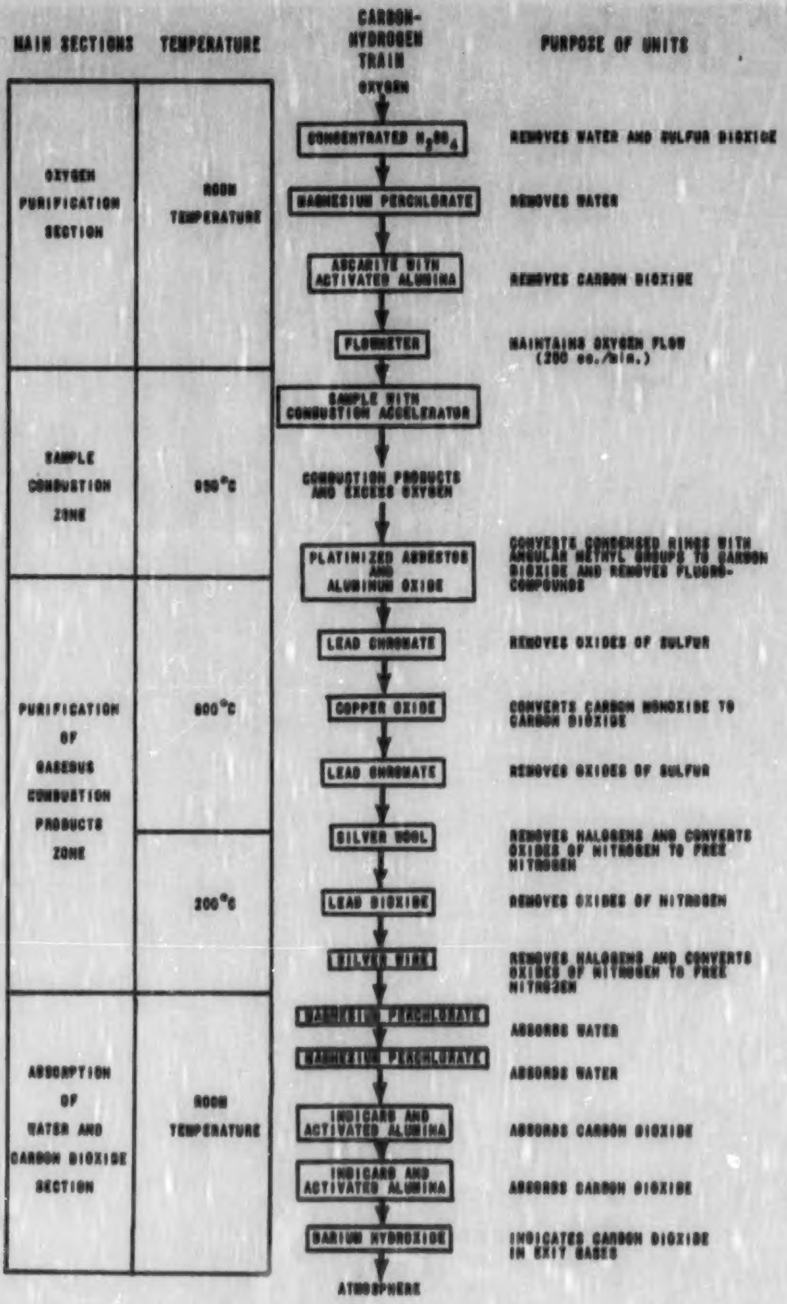
MAIN SECTIONS
TEMPERATURE
CARBON-HYDROGEN TRAIN
PURPOSE OF UNITS
OXYGEN PURIFICATION SECTION
ROOM TEMPERATURE
SAMPLE COMBUSTION ZONE
850°C
PURIFICATION OF GASEOUS COMBUSTION PRODUCTS ZONE
800°C
200°C
ABSORPTION OF WATER AND CARBON DIOXIDE SECTION
ROOM TEMPERATURE
OXYGEN
CONCENTRATED H_2SO_4
REMOVES WATER AND SULFUR DIOXIDE
MAGNESIUM PERCHLORATE
REMOVES WATER
ASCARITE WITH ACTIVATED ALUMINA
REMOVES CARBON DIOXIDE
FLOWMETER
MAINTAINS OXYGEN FLOW (200 cc./min.)
SAMPLE WITH COMBUSTION ACCELERATOR
COMBUSTION PRODUCTS AND EXCESS OXYGEN
PLATINIZED ASBESTOS AND ALUMINUM OXIDE
CONVERTS CONDENSED RINGS WITH ANGULAR METHYL GROUPS TO CARBON DIOXIDE AND REMOVES FLUORO-COMPOUNDS
LEAD CHROMATE
REMOVES OXIDES OF SULFUR
COPPER OXIDE
CONVERTS CARBON MONOXIDE TO CARBON DIOXIDE
LEAD CHROMATE
REMOVES OXIDES OF SULFUR
SILVER WOOL
REMOVES HALOGENS AND CONVERTS OXIDES OF NITROGEN TO FREE NITROGEN
LEAD DIOXIDE
REMOVES OXIDES OF NITROGEN
SILVER WIRE
REMOVES HALOGENS AND CONVERTS OXIDES OF NITROGEN TO FREE NITROGEN
MAGNESIUM PERCHLORATE
ABSORBS WATER
MAGNESIUM PERCHLORATE
ABSORBS WATER
INDICARB AND ACTIVATED ALUMINA
ABSORBS CARBON DIOXIDE
INDICARB AND ACTIVATED ALUMINA
ABSORBS CARBON DIOXIDE
BARIUM HYDROXIDE
INDICATES CARBON DIOXIDE IN EXIT GASES
ATMOSPHERE

Figure 2. General outline of carbon-hydrogen train.

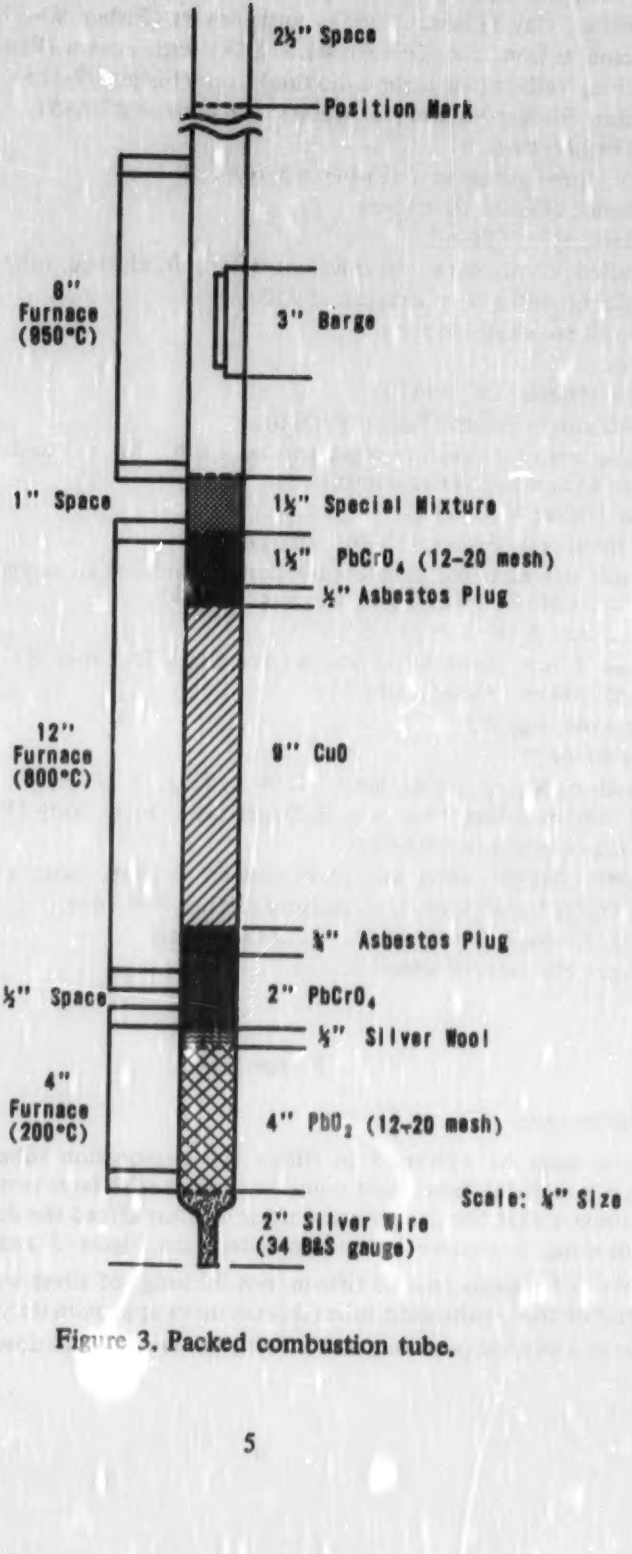
2½" Space
Position Mark
8" Furnace (850°C)
3" Barge
1" Space
1½" Special Mixture
1½" $PbCrO_4$ (12-20 mesh)
½" Asbestos Plug
12" Furnace (800°C)
9" CuO
¾" Asbestos Plug
½" Space
2" $PbCrO_4$
½" Silver Wool
4" Furnace (200°C)
4" PbO_2 (12-20 mesh)
Scale: ¼" Size
PACKED COMBUSTION TUBE
2" Silver Wire (34 B&S gauge)

Figure 3. Packed combustion tube.

5. Baskets, test tube, one size C, four size D (Fisher #14-966)
6. Boats, combustion, clay (Fisher #7-651) with covers (Fisher #7-652)
7. Boats, large, combustion, clay (Fisher #12-183N) with covers (Fisher #12-1835 N)
8. Bulbs, absorption, Nesbitt (for carbon dioxide), four (Fisher #7-515)
9. Bulbs, absorption, Stetser-Norton (for water), two (Fisher #7-565)
10. Charger-rake (Fisher #7-610)
11. Clamps, utility, three-prong grip (Fisher #5-768-10)
12. Desiccator cabinet (Fisher #8-645-5)
13. Erlenmeyer flask, two, 500-ml
14. Furnace, modified, combustion, organic, multiple unit, electric, tube type (Lindberg #123-T-S)
15. Furnace, muffle, operating temperature of 950 C
16. Glass tubing to fit two-hole stoppers
17. Gloves, lint-free
18. Inserter, sample (Dietert Co. #3452)
19. Jar, drying, 300 mm in height (Fisher #9-210)
20. Oxygen cylinder with (a) pressure regulator, adjustable from 0 to 10 lb of pressure on the low pressure side and (b) needle-valve control
21. Pan, enameled (Fisher #9-017)
22. Pan, stainless steel, two (Fisher #13-361, size D)
23. Rotameter, ends designed for tube connections, maintains an oxygen flow of 250 cc per min (Ace Glass, Inc., Rota-Kit, Tru-Taper #1, size 1A-15-1)
24. Stand, support, size A (Fisher #14-675)
25. Stopcock, glass, T-bore, 8mm O. D. stems (Ace Glass, Inc. #8178)
26. Stopcock, hard rubber (Fisher #14-630)
27. Stoppers, two-hole, size #7
28. Timer (Fisher #6-662)
29. Tube, combustion, Vycor, 38 in. long, 1 1/4 in. I. D., 1 1/2 in. O. D., one end tapered to fit 3/16 in. I. D. rubber tubing (may use McDanel tube, 36 in. long [Fisher #CTT11436])
30. Tube cleaners, size A (Fisher #3-642)
31. Tubing, bubble, Argyle universal, plain, lumen 3/16-in. bore (Aloe Scientific #AR-500)
32. Tubing, rubber, red, thin wall, 3/16-in. bore (Fisher #14-166)
33. Tubing, Tygon, 1/4-in. I. D., 3/8-in. O. D., 1/16-in. wall
34. Washers, Milligan gas, two, (Fisher #7-513)
35. Yardstick

Preparation

Filling the combustion tube.

Considerable care must be exercised in filling the combustion tube. The gaseous combustion products diffusing through the tube must come in contact with large surface areas, but the materials must not be so compact that the gas pressure heads will not afford the desired gas velocities through the tube. The following procedure is recommended (see Figure 3 and the section on reagents):

a) Twist a number of strands (ten to fifteen, 6-8 in. long) of silver wire together and insert into the tapered end of the combustion tube (this occupies approximately 2 in. of the tube).

b) Hold the tube in a vertical position, that is, with the tapered end down.

c) Add approximately 100 g lead dioxide to the tube. The layer should be 4 in. deep (a yardstick may be helpful).

d) Insert 10 g silver wool to form a 1/2-in. layer.

e) Add approximately 50 g lead chromate to form a 2-in. layer (mix in a small amount of asbestos to help prevent caking).

f) Insert asbestos loosely to form a 3/4-in. plug.

g) Add approximately 500 g cupric oxide to form a 9-in. layer.

h) Insert asbestos loosely to form a 1/2-in. plug.

i) Add approximately 37 g lead chromate to form a 1 1/2-in. layer (again mix in a small amount of asbestos).

j) Add the special mixture consisting of 20 ml of platinized asbestos plus 20 ml of asbestos plus 10 ml of aluminum oxide (approximate unpacked volume measurements). The layer should be 1 1/2 in. deep.

Assembling the combustion train.

The components of the apparatus are assembled in the following sequence:

a) Oxygen cylinder with attached regulator that will afford a delivery pressure of 10 (p. s. i. g.)

b) Hard rubber stopcock with on-off valve

c) Erlenmeyer flask, 500-ml, with two-hole, size #7 stopper (serves as a liquid-backflow trap)

d) Gas washer containing 150 ml concentrated sulfuric acid

e) Drying jar: Magnesium perchlorate is placed in the lower half and ascarite, topped with activated alumina, in the upper half. A small layer of glass wool is inserted beneath the magnesium perchlorate and above the activated alumina. The oxygen flow into the jar is through the bottom inlet.

f) Rotameter, with the stainless steel float at a level affording an oxygen flow of 250 cc per min

g) Glass stopcock held by a utility clamp on a support stand

h) Combustion tube with sample inserter and oxygen baffle attached at the non-tapered end; a position mark, etched on the outside of tube 2 1/2 in. from the non-tapered end to enable the analyst to keep the tube in correct position within the furnace units; aluminum foil placed around the tapered end of the tube to maintain heat inside the tube and prevent water vapor from condensing

i) Two Stetser-Norton bulbs (for water absorption) set up in series; each contains magnesium perchlorate between two loose layers of glass wool

j) Two Nesbitt bulbs (for carbon dioxide absorption) assembled in series: a 1/2-in. layer of glass wool is placed in the bottom of the bulb. Indicarb is then added to a point 3/4-in. from the shoulder of the bulb. The Indicarb is covered by a 1/4-in. layer of activated alumina. Introduce sufficient glass wool to reach the neck area and cotton in hollow stopper. (Note: For highly combustible materials or materials with high carbon content, more than two Nesbitt bulbs may be necessary.)

k) Erlenmeyer flask, 500-ml, with two-hole, size #7 stopper (serves as a liquid-backflow trap)

l) Gas washer, containing 150 ml of barium hydroxidethymolphthalein solution

m) Tygon tubing as connections between the oxygen cylinder, stopcock, erlenmeyer flask, gas washer, and drying jar

n) Argyle tubing, used between drying jar, rotameter, glass stopcock, and the sample inserter

o) Rubber tubing, between the tapered end of the combustion tube, absorption bulbs, second Erlenmeyer flask trap, and final gas washer.

Conditioning the combustion tube.

A freshly packed tube contains excess moisture and must be dried out for 2 hr as directed in step 1 in the following "Procedure." After the 2-hr period, the analyst continues with the remaining procedural steps.

If the combustion tube has been previously used but has remained idle for more than 1 day, the analyst conditions the tube by performing only steps 2 and 3. During this idle period, the glass stopcock must be turned so that nothing can flow into the combustion tube. The rubber stopcock is likewise closed so that the sulfuric acid does not flow from the gas washer back into the trap.

If the stopcocks are at their proper shut-down positions, no conditioning of the combustion tube is necessary with an idle period of 1 day or less.

A conditioned combustion tube in continuous use should last for about 6 months or approximately 700 analyses. A close estimate of the life span for a conditioned tube is impossible to determine because of the variation in the samples' impurities and carbon-hydrogen concentrations.

Note: Before performing the following conditioning procedure, review the start-up and shut-down procedures.

	Procedure		Comments
1.	With the 8-in. furnace at 950 C ± 20 C (control level about 8.9), the 12-in. furnace at 800 C ± 20 C (about 4.5), and the 4-in. furnace at 200 C ± 10 C (about 2.0), allow oxygen to flow through the combustion train at 250 cc per min, with the rubber and glass stopcocks open. (Do not connect absorption bulbs.) NOTICE: For newly packed tubes, wait 2 hr before starting the next step.	1.	a) Putting the temperature knobs of the 8-in. and the 12-in. furnaces on "high" and the 4-in. furnace on 3 will allow the heat-up time to be about only 35 min. b) CAUTION: Never allow the temperature to exceed 1,000 C for the 8-in. furnace, 840 C for the 12-in. furnace, and 220 C for the 4-in. furnace. c) The stainless steel float in the rotameter should be approximately at the 8.2-cm level (the setting established for oxygen gas).
2.	Follow the procedure outlined for analyzing samples except use 0.2 to 0.5 g of A. C. S. grade sucrose without a combustion aid.	2.	a) Sucrose must be previously dried at 110 C for 1 hr. b) See section on Samples. c) CAUTION: Higher weights of sucrose combust too violently.
3.	Repeat step 2 above if observed carbon and hydrogen contents do not agree with theoretical values.	3.	This step is usually necessary only with a freshly packed tube.

REAGENTS

All reagents are of ACS analytical reagent-grade quality. All solutions are prepared using distilled or deionized water. Reagents are as follows:

1. Oxygen, 99.5 percent pure (the oxygen should be prepared from liquid air since oxygen prepared by electrolysis contains traces of hydrogen)
2. Sulfuric acid, concentrated
3. Dehydrite (anhydrous magnesium perchlorate)
4. Ascarite (sodium hydroxide on asbestos), 8-20 mesh.
5. Activated alumina, indicating, 8-14 mesh (Fisher #A-545)
6. Silver wire, 34 B & S gauge, pure grade (Fisher #20-272)
7. Lead dioxide, brown, 12-20 mesh
8. Silver wool
9. Lead chromate, 12-20 mesh; or powder which has fused at 820 C for 1 hr and ground to about 12-20 mesh
10. Asbestos, medium or long fiber, acid-washed
11. Cupric oxide, black wire; before using, ignite at 800 C for 1 hr
12. Platinized asbestos, 5 percent (Fisher #P-152)
13. Aluminum oxide, anhydrous
14. Accelerator chips, Combax (iron) (Fisher #C-420)
15. Indicarb, 6-10 mesh (Fisher #1-181)
16. Absorbent cotton
17. Glass wool
18. Stopcock grease, Kel-F 90 (Fisher #14-635-10)
19. Barium hydroxide solution: dissolve 12.0 g $Ba(OH)_2$ in distilled water and dilute with same to 1 liter
20. Thymolphthalein solution: dissolve 10.0 g thymolphthalein in ethanol and dilute with same to 1 liter
21. Barium hydroxide-thymolphthalein solution: add 0.5 ml of the thymolphthalein solution to 150 ml of the barium hydroxide solution

SAFETY PRECAUTIONS

A wooden safety shield 2 ft x 2 ft x 3/8 in., or the equivalent, should be placed about 3 ft from the right side of the furnace base, such that if the rubber stopper with the sample inserter should blow out, the stopper and inserter would hit the shield.

The sample boats should never be inserted faster than the prescribed 1 in. each 5 min after the initial 5-min wait, for the first 20 min of total insertion time. Faster sample insertions may cause the above mentioned blow-out or a more violent explosion. If the sample ignition is too violent, the analyst should divert the oxygen flow by turning the glass stopcock and allowing the oxygen to flow into the room.

Normally the oxygen flow rate is reduced during the initial ignition (which generally occurs on the first or second 1-in. insertion) and should not be adjusted during this period. The flow rate of 250 cc per min is sufficient to prevent the back flow of gases into the rotameter. Always keeping the sample inserter's blunt end, while in initial position, approximately 1/4 in. away from the baffle will help maintain the oxygen flow.

If the flow of exit gas stops, the oxygen inlet flow is immediately turned off by using the glass stopcock, as mentioned above. This gas-flow stoppage may be due to an unopened absorption bulb or too tight tube compaction.

The analyst should always be aware of the hot boats, the hot furnace parts, and the general bubble flow-rate in the exit gas washer. He should not remain close to the combustion tube during the sample's initial ignition because possible explosions are anticipated mainly at this time.

SAMPLE PREPARATION

The details of sample preparation procedures that describe the drying and grinding techniques are not discussed here. In general, raw refuse, residue (organics), and compost are dried at 70 C to a constant weight and ground to a particle size of less than 1 to 2 mm. Residue (fines) and fly ash samples are dried at 105 C to a constant weight and pulverized to pass through a #60 sieve.

All samples must be redried no more than one week before being weighed for the analyses. Those samples originally dried at 70 C must be redried at 70 C for 4 hr; those samples originally dried at 105 C must be redried at 105 C for 1 hr. After redrying procedures, all samples must be kept in tightly closed containers and in a desiccator until the analyses are performed.

PROCEDURE

Start-Up

The start-up procedure for the carbon-hydrogen analyses is always the same, except for the use of a freshly packed tube, as previously mentioned. The following procedure outlines the routine steps in preparing the carbon-hydrogen train for the analyses.

	Procedure		Comments
1.	Set the temperature controls of the 8-in. and 12-in. furnaces on high and that of the 4-in. furnace on 3 for about 35 min. Final temperatures for the 8, 12, and 4-in. furnaces are 950 C ± 20 C, 800 C ± 20 C, and 200 C ± 10 C, respectively.	1.	CAUTION: Never allow the temperature to exceed 1,000 C for the 8-in. furnace, 840 C for the 12-in. furnace, and 220 C for the 4-in. furnace.
2.	After each furnace has reached its appropriate temperature, set the 8-in. furnace control on 8.9, the 12-in. furnace control on 4.5, and the 4-in. furnace control on 2.0.	2.	These numbers are not absolute and may change with the age of the elements.
3.	Open the rubber stopcock and without much delay allow the oxygen to flow through at approximately 250 cc per min. The delivery pressure gauge is set at 10 (p. s. i. g.). Flow adjustments are made with the needle-valve control.	3.	a) The glass stopcock should be open to the room. b) If the oxygen is not soon allowed to flow, sulfuric acid will flow back into the trap. c) The stainless steel float in the rotameter should be set and maintained at approximately the 8.2-cm level.
4.	Turn the glass stopcock to allow oxygen to flow through the combustion tube.	4.	a) If the float drops drastically toward the zero level, immediately return glass stopcock to its former position. Then the

			charger-rake rod may be used to loosen the tube packing. b) Absorption bulbs should not be attached.
5.	Attach at least two of each type of absorption bulbs and again notice the stainless steel float and bubbles in the exit-gas washer.	5.	a) The bubble flow rate in the exit-gas washer is a good indication as to how the oxygen is flowing through the train. If the bubbles do not start, an absorption bulb may be closed or packed too tightly. b) If the samples to be analyzed are known or suspected to contain more than 30 percent carbon, and if sample weights of more than 1.5 g are used, attach three or more absorption bulbs for the CO_2 collection.
6.	Adjust the oxygen flow to be approximately 250 cc per min.; the stainless steel float should be at the 8.2-cm level.	6.	The flat end of the sample inserter should always be slightly away from the end of the baffle to allow the free flow of oxygen.
7.	With the train completely assembled, allow the oxygen to flow through the system for 10 min.	7.	This step ensures the conditioning of the absorption bulbs.
8.	Remove the absorption bulbs from the train and close each bulb to the atmosphere.		
9.	Determine and record the weight of each CO_2-absorbing bulb and each H_2O-absorbing bulb. These weights represent the initial weights of the absorption bulbs.	9.	a) The bulbs should be near room temperature before being weighed. Normally, each bulb will be near room temperature if the order of weighing is started with the last bulb in the train. b) Before weighing, each bulb is momentarily vented to the atmosphere and wiped clean with a lint-free cloth. c) Use an analytical balance with a 200-g capacity and 0.1 mg readability. d) Weights are recorded to the nearest 0.0001 g.
10.	After opening the absorption bulbs to permit gas flow, quickly return the bulbs to the train assembly.	10.	Start with the bulb furthest from the furnace. The connection of the bulbs to the furnace should be performed last.
11.	When the gas starts through the exit-gas washer, the train is ready for sample analyses.	11.	If the gas does not start through the exit-gas washer, check bulbs, openings, and compaction of absorbing materials.

Blanks

Increase in weight of the absorption bulbs is due to 1) sample ignition, 2) sample container's contamination, 3) atmospheric contamination during sample insertion, and 4) impure oxygen gas. The blank analyses determine the effects of all the above factors except sample ignition on the weights of absorption bulbs. These blank analyses are conducted like the regular sample analyses, except that preignited, empty sample containers with covers and an unpacked combustion tube are employed. The unpacked tube is needed for the blank analyses because lead dioxide is highly hygroscopic.

The analyst is advised to perform triplicate observations and record the humidity (which may be estimated) of the room during the analyses. Whenever the oxygen supply is changed or the room humidity greatly changes, the blank analysis should be repeated. The carbon dioxide blank value is usually zero and the water blank value normally varies with analysts.

Standards

After conditioning the combustion tube as previously described, the analyst should periodically check the carbon-hydrogen train by analyzing standards such as sucrose, naphthol, urea, graphite, calcium carbonate, and solid waste samples that have been previously analyzed. The procedure for analyzing standards is the same as described for sample analyses. Analyze, however, only 0.2 to 0.5 g of sucrose, and without a combustion aid, since this standard burns quickly and easily. Benzoic acid, although commonly used as a carbon standard, reacts too violently for this method.

Samples

At this point, all the necessary conditioning should have been performed so that the carbon-hydrogen train is now ready for sample analyses. The following procedure applies to all solid waste materials, and also to blanks and standards with the previously mentioned changes. All the sample containers and covers employed in the method must have been previously ignited at 950 C for 1 hr, then cooled and stored in a desiccator until used.

The analyst is advised to analyze samples of each particular type of solid waste material as a unit. Switching back and forth between different types of materials (which usually vary greatly in their carbon-hydrogen content) causes unnecessary reconditioning and rechecking of the combustion tube. To ensure good results, the analyst should always analyze a standard before analyzing a particular type of solid waste material. This standard should be of the same general character as the type of material to be analyzed.

A combustion aid must be mixed with each sample after the sample has been weighed into its container. Some samples combust vigorously and the sample injection procedure may have to be slowed down; however, even vigorously reacting samples need a combustion aid.

The analyst must use his own judgment in the selection of the sample container, the number of absorption bulbs in the train, the unscheduled removal of an absorption bulb, and the change of the sample insertion procedure.

Procedure	Comments
1. Transfer at least 1 to 2 g of a sample into each of two previously weighed combustion boats. Determine and record the weight of each sample to the nearest 0.0001 g.	1. a) Sample weights up to 10 g may be used if non-uniformity of sample warrants. b) Fluffy samples such as compost samples require the larger containers. c) Minimize handling of boats and lids to prevent contamination. d) Keeping the boats in a particular order, or numbering them, will prevent mix-up of samples.

	e) DO NOT pellet any sample. If a 1- to 2-g sample will not fit into a particular boat, use a larger container.
2. Sparsely sprinkle each sample with a few iron chips. Using a spatula, mix the combustion aid throughout the sample.	2. a) Granular tin (Fisher #12-173) may be used as an accelerator if iron chips are not available. b) REMINDER: DO NOT use a combustion aid with sucrose.
3. Store each boat (containing a sample) and its lid in a desiccator until they are transferred to the combustion tube.	3. a) It is convenient to use a stiff asbestos pad to support the boats while in the desiccator and during transfer from one place to another. b) The desiccator must contain CO_2-absorbing materials.
4. Cover each boat with its lid and transfer them from the desiccator to insertion end of combustion tube. IMMEDIATELY go to the next step.	4. REMINDER: Flat end of sample inserter must be slightly away from the baffle to permit free flow of oxygen.
5. Remove stopper with attached sample inserter and place boat about half way into tube. Then close the combustion tube by moving the sample inserter, with baffle, along the bottom of the tube; holding the tube with the left hand, twist the stopper tightly into the tube. IMMEDIATELY go to next step.	5. a) Do this step quickly. The blank value will only be applicable if the time required for placing the boat in the combustion tube is constant. b) If the combustion tube contains the boat and lid from the previous sample analyses, use the charger-rake to remove the boat and lid onto a stiff asbestos pad. Then drop the boat and lid into an enameled pan lined with asbestos pads. After they have cooled, the boat and lid are transferred to a stainless steel pan, cleaned, and finally ignited at 950 C for 1 hr before being reused.
6. Upon closing the combustion tube, set a timer for 60 min. Check the flow rate to see if it is 250 cc per min (stainless steel float at 8.2-cm level) after the gases are bubbling through the exit-gas washer.	6. a) The stainless steel float will drop momentarily and adjustment is needed if it remains below the 7.1-cm level for more than a few moments. DO NOT, however, attempt to adjust the flow-rate if the sample ignition has started. Except when using the large clay boats, sample ignition usually starts after the sample is closer to the 950 C zone. b) SAFETY REMINDER: If bubbles in exit-gas washer stop completely for more than 5 to 10 min and the stainless steel float begins dropping (caused by too vigorous sample combustion), turn the glass stopcock, thus diverting the oxygen from the combustion tube to the room. Whenever the stopcock must be turned, the results of that particular test must be discarded.

7. After 5 min, move the sample boat 1 in. toward the 950 C zone.	7. If the sample has already ignited vigorously, delay this step for another 5 min.
8. At the end of each of 3 successive 5-min intervals, move the sample boat 1-in. toward the 950 C zone.	8. a) DO NOT move the sample boat at a faster rate, even if one of the 1-in. insertion steps was forgotten. After some experience, however, the analyst may accelerate the insertion procedure when analyzing residue (fines) or fly ash samples. b) During this step, the Indicarb may indicate that another tared absorption bulb should be added.
9. After another 5 min, move the sample boat completely into the middle of the 950 C zone.	9. a) The entire period of sample insertion should not exceed 30 min. b) If the large clay boats were employed, most of the boat will be beyond the center of the 950 C zone.
10. Allow the oxygen to flow through the train for the rest of the 60-min period.	
11. Remove the absorption bulbs from the train and close each bulb to the atmosphere.	11. The oxygen continues to flow through the combustion tube while the absorption bulbs are removed. If more than 30 min will elapse before another sample is started, however, the oxygen flow must be diverted away from the combustion and into the room by turning the glass stopcock.
12. Determine and record the weight of each CO_2-absorbing bulb and each H_2O-absorbing bulb.	12. a) This weight represents the final weight of each absorption bulb and is used as the initial weight of each bulb for the next sample. b) The time required for weighing should not exceed 15 min.
13. Return the sample inserter to the start position.	13. When the flat end of the sample inserter is slightly away from the baffle, thus permitting a free flow of oxygen through the baffle, the sample inserter is in the start position.
14. If another sample, contained within a boat, is ready to be analyzed, repeat the procedure starting with step 4.	
15. If the train is not to be used for more than 1/2 hr, see "Shut-Down" procedure.	

Shut-Down

As mentioned in the section on conditioning the combustion tube, the proper shut-down procedure prevents the necessity for reconditioning the combustion tube. If the train is not used for more than 1/2-hr and the oxygen is allowed to flow through the combustion tube, the packing materials (especially the lead dioxide) will dry out and require reconditioning before the train can be used again. If the train is to be used the same day but more than 30 min will elapse, the furnaces' temperatures may be maintained, but the oxygen flow must either be diverted from flowing through the combustion tube or shut off completely. Whenever the oxygen flow is off, the glass and rubber stopcocks must be closed as described below.

Procedure	Comments
1. Disconnect the absorption bulbs from the combustion tube and close each one to the atmosphere.	1. Any remaining boat and lid should be removed from the combustion tube.
2. Having the combustion tube stoppered and the sample inserter in the start position, turn each furnace off.	2. Furnaces need not be turned off if used later the same day.
3. Turn off the oxygen flow first at the main regulator valve on the oxygen cylinder, then at the low pressure valve. IMMEDIATELY go to the next step.	
4. Turn the glass stopcock to divert any oxygen flow from the combustion tube to the room. IMMEDIATELY go to the next step.	
5. Close the rubber stopcock.	5. When the rubber stopcock is closed, the backflow of sulfuric acid into the trap is prevented.

CALCULATIONS

Standards

Formula.

Employ the following formula to calculate the theoretical concentration of either carbon or hydrogen in a standard sample:

$$\%E = \frac{(N)\,(F)\,(100)}{(S)\,(P)}$$

where

% = the percent by weight

E = the element, either carbon or hydrogen

N = the number of atoms of the element in a molecule of the standard

F = a factor, derived by dividing the gram-atomic weight of the element by the gram-molecular weight of the standard

S = the weight of the total sample

P = the decimal fraction representing the concentration of the standard compound in the total analyzed sample. (Note: this decimal fraction is the only fraction containing the component for which the sample is being analyzed.)

Example.

Pure Sucrose, $C_{12}H_{22}O_{11}$ (NBS Grade)

$$\%C = \frac{(12)\left(\frac{12.01}{342.30}\right)(100)}{(1.0000)\ (1.00)} = 42.10$$

$$\%H = \frac{(22)\left(\frac{1.008}{342.30}\right)(100)}{(1.0000)\ (1.00)} = 6.48$$

When the impurities listed on the bottle are considered, the calculated percents of carbon and hydrogen in ACS grade sucrose are 42.09 and 6.48 respectively.

Samples

Carbon.

Employ the following formula to calculate the concentration of carbon in a solid waste sample:

$$\%C = \frac{(A - B)\ (X)\ (100)}{(S)}$$

where

% = the percent by weight

C = the element carbon

A = the sum total increase in the weight of the CO_2-absorbing bulbs as determined in the unknown analysis

B = the sum total increase in the weight of the CO_2-absorbing bulbs as determined in the blank analyses

X = a factor, derived by dividing the gram-atomic weight of carbon by the gram-molecular weight of carbon dioxide (i. e., (12.01) ÷ (44.01) = 0.2729)

S = the weight of the total sample

Hydrogen.

Employ the following formula to calculate the concentration of hydrogen in a solid waste sample:

$$\%H = \frac{(A - B)\ (Y)\ (100)}{(S)}$$

where

% = the percent by weight

H = the element hydrogen

A = the sum total increase in the weight of the H_2O-absorbing bulbs as determined in the unknown analysis

B = the sum total increase in the weight of the H_2O-absorbing bulbs as determined in the blank analyses

Y = a factor, derived by dividing the gram-molecular weight of hydrogen by the gram-molecular weight of water (i. e., $(2.016) \div (18.02) = 0.1119$)

S = the weight of the total sample

METHOD EVALUATION

The accuracy of this newly developed method is established in Table 1. This method can analyze solid waste materials containing various forms of carbon to within one actual percent of the true value. For these tests, sucrose, naphthol, and urea were selected to represent hydrocarbons. Graphite was employed as an elemental form of carbon, and calcium carbonate as an inorganic form. This method has accurately analyzed (100.00% recovery) specially prepared residue (fines) samples calculated to contain only 0.46 percent carbon and 0.01 percent hydrogen. This method should definitely not be employed to analyze samples that contain less than 0.1 percent carbon.

The precision of this method was determined by analyzing in triplicate a number of solid waste samples of various types. The pooled standard deviation of the observations for each type of solid waste was calculated using an Olivetti Underwood Programma 101. The calculations revealed that in the analyses of each type of waste, the duplicate and triplicate determinations were equally precise (Table 2). To ensure precision, the particle size of the samples must be less than 2 mm or passed through a #60 sieve, then thoroughly mixed before analyzing.

With this macro method, the analyst normally uses a 1- to 2-g sample, but he is not restricted to this amount. Because of the difficulties in preparing a very uniform sample, sample weights below 1 g have been found inadequate when analyzing solid waste materials. But samples up to 10 g have been analyzed with no difficulty. The extra sample weights, however, add little to the precision of this method.

TABLE 1
ACCURACY OF CARBON AND HYDROGEN DETERMINATIONS OF STANDARD COMPOUNDS

Compound	Number of determinations	% Element calculated		% Element found		% Recovery	
		C	H	C	H	C	H
Sucrose, NBS	6	42.10	6.48	42.07	6.39	99.93	98.61
Sucrose, ACS	6	42.09	6.48	42.02	6.39	99.83	98.61
1-Naphthol, ACS	3	83.31	5.59	82.72	5.86	99.29	104.83
Urea	3	19.99	6.71	19.38	6.66	96.95	99.25
Calcium carbonate	3	11.97	---	12.04	---	100.58	---
Graphite*	3	83.28	---	84.01	---	100.88	---

*Since the graphite is not pure, the graphite, for this study, was ignited in air at 1,150 C to determine the percent of ash impurities.

TABLE 2
STANDARD DEVIATION OF THE CARBON AND HYDROGEN DETERMINATION ON SUCROSE AND SOLID WASTES

Type of sample	Number of samples	Standard deviation*			
		Carbon observations		Hydrogen observations	
		Duplicates	Triplicates	Duplicates	Triplicates
Sucrose, NBS	2	---	0.17	---	0.15
Sucrose, ACS	2	---	0.15	---	0.19
Compost	26	---	0.29	---	0.10
Compost	56	0.22	---	0.14	---
Raw garbage	17	0.18	0.19	0.19	0.18
Residue:					
Fines	16	0.04	0.06	0.04	0.03
Organics	8	0.21	0.23	0.22	0.18
Fly ash	9	0.04	0.08	0.06	0.04

*A variance estimate can be calculated from the duplicate (or triplicate) set of observations for each sample. The pooled variance is essentially an average of all such estimates for samples of a given type. It is assumed that a single underlying variance exists for all samples of a given type. The pooled variance is then the best estimate of this underlying variance. The pooled standard deviation is the square root of the pooled variance and is used to estimate the underlying standard deviation.

ACKNOWLEDGMENTS

The author wishes to thank the Office of Solid Waste Management Programs for providing samples from incinerators, and Israel Cohen, Research Services Laboratory, Solid Waste Research Laboratory, for preparing many of these samples.

The author gratefully acknowledges the contribution of personnel at the PHS-TVA Compost Plant, Johnson City, Tennessee, who collected and prepared most of the compost samples used in developing this method.

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LABORATORY PROCEDURES TO DETERMINE THE NITROGEN CONTENT OF SOLID WASTES

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INTRODUCTION

Discussion

Nitrogen becomes a significant solid waste parameter* when it is evaluated in conjunction with two other parameters – carbon and hydrogen. The change in the C/N ratio of compost can be used to determine (a) the degree and rate of biological decomposition of organic matter in the compost (1,2) and (b) the suitability of the final product for use in agricultural soils (3). A knowledge of the nitrogen, carbon, and hydrogen contents of an incinerator's load and combustion products can also enable the engineer and scientist to (a) calculate the theoretical air requirements and combustion products, (b) formulate appropriate material and energy balance equations, and (c) evaluate and control effectively the efficiency of an incinerator system (4).

To ensure the precise and accurate determination of the nitrogen content of solid wastes, investigations of the applicability of existing analytical procedures were conducted in our laboratory. Analyses of solid waste samples containing up to 8 percent nitrogen have demonstrated that the Kjeldahl-Wilfarth-Gunning-Winkler method, the comprehensive nitrogen method, and the automated Dumas method (as employed in the Coleman Nitrogen Analyzer) may be used in the characterization of solid wastes (5). Detailed descriptions of these three methods are presented in the following sections.

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KJELDAHL-WILFARTH-GUNNING-WINKLER METHOD

Discussion

The Kjeldahl-Wilfarth-Gunning-Winkler method† (1–5) may be employed to characterize solid waste samples if their nitrogen content is primarily organic and/or ammoniacal. Since the method will not recover nitrate nitrogen quantitatively, its applicability is presently limited to the analysis of municipal refuse, unfortified compost, incinerator residue, and other samples with little, if any, nitrate content. (Either the comprehensive nitrogen method or the automated Dumas method should be employed to determine the total nitrogen content of samples containing significant quantities of nitrate, e. g. fortified compost, or samples of unknown nitrate concentration.)

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*In this paper, the term parameter denotes a variable or characteristic of interest.

†See Appendix for a brief history of the Kjeldahl Method.

In the Kjeldahl-Wilfarth-Gunning-Winkler method, a sample is first digested with sulfuric acid. To increase the speed of the reaction and ensure the digestion of substances whose decomposition temperatures are above the boiling point of sulfuric acid, mercuric oxide and potassium sulfate are added. After the digestion has been completed, the mixture is then treated with alkaline sodium thiosulfate, which destroys the mercuro-ammonium complex and permits the distillation of ammonia into 4-percent boric acid. A titration of the ammonium borate with standard sulfuric acid then permits the calculation of the total organic and ammoniacal nitrogen content of the sample.

Safety Precautions

1. Safety glasses should always be worn by the analyst while handling concentrated sulfuric acid and digestion mixtures.
2. The analyst should exercise care in weighing, transferring, and disposing mercuric oxide, since inhalation, ingestion, or contact with the compound may cause mercurial poisoning.
3. Exhaust hoods (or special traps) should be employed during sample digestion to minimize the analyst's exposure to the evolving fumes.

Equipment

1. Apparatus, Kjeldahl, digestion and distillation with (a) a 6-heater unit, either gas or electric, capable of bringing 250 ml of water at 25 C to a rolling boil in 5 min, (b) block tin condensers enclosed in a copper coated cooling tank, and (c) Pyrex delivery tubes (individual Pyrex condensers and appropriate adapters may be substituted).
2. Balance, analytical, 0.1-mg readability
3. Bottle, dropper, Pyrex, 250-ml
4. Bottles, reagent, Pyrex, 1-liter and 2-liter
5. Bulbs, connecting, cylindrical or spherical trap style, Pyrex, 5 to 6 cm in diameter
6. Buret, Pyrex, 25 ml, graduated in 0.1-ml divisions
7. Carrier designed to hold six 800-ml Kjeldahl digestion flasks (Fisher Scientific Co., Catalogue #10-114)
8. Desiccator, large, either Pyrex or stainless steel cabinet type
9. Dispensers, delivery head with ₸ 24/40 joint, 25-ml and 40-ml (available from California Laboratory Equipment Company, 1165 67th Street, Oakland, California 94608)
10. Flasks, Erlenmeyer, Pyrex, with ₸ 24/40 mouth, 500-ml
11. Flasks, Erlenmeyer, Pyrex, wide-mouth, 500-ml
12. Flasks, Kjeldahl, for digestion and distillation, Pyrex, 800-ml
13. Hood, exhaust and special stack to outside for venting fumes during the digestion of samples
14. Stoppers, rubber, size 7, for connecting bulbs to digestion flasks
15. Tubing, rubber, thin wall, for connecting bulbs to condensers and cooling tank to cold water supply and sink drain

Reagents

The chemicals in the following list should all be reagent grade and nitrogen-free.

1. Sucrose
2. Acetanilide
3. Disodium ethylenediaminetetraacetic acid (disodium EDTA)

4. Hengar granules, plain
5. Potassium nitrate
6. Mercuric oxide
7. Sulfuric acid, concentrated (95 to 98 percent)
8. Silicone antifoaming agent: General Electric No. 66 or Dow Corning Antifoam Q
9. Zinc metal, granulated
10. Alkaline thiosulfate solution: Dissolve 450 g sodium hydroxide in approximately 700 ml distilled water, cool, add 80 g sodium thiosulfate ($Na_2S_2O_3.5H_2O$), mix, and dilute with distilled water to 1 liter.
11. Boric acid solution: Dissolve 40 g boric acid in distilled water and dilute with same to 1 liter.
12. Methyl purple solution (indicator): Dissolve 0.3125 g methyl red and 0.2062 g methylene blue in distilled water (or 0.1 percent ethyl alcohol) and dilute with same to 250 ml.
13. Sulfuric acid standard solution, 0.1 N: Dissolve approximately 3 ml of concentrated sulfuric acid in 800 ml distilled water and dilute with same to 1 liter. Determine the normality of the solution using a primary standard such as sodium borate.

Technique Evaluation

Before initiating the characterization of solid waste samples, the investigator should evaluate his technique by analyzing compounds of known nitrogen content. Either 0.25 g acetanilide or 0.5 g disodium EDTA may be used as a standard sample (see section on Accuracy and Precision).

Solid Waste Sample Preparation

A solid waste sample must undergo physical preparation before its characterization is initiated in the laboratory. First it must be dried to constant weight, preferably in a forced-air or mechanically convected oven. A temperature of 70 to 75 C should be used to dry municipal refuse or compost; incinerator residue may be dried at 100 to 105 C. The particle size of the dried sample should then be reduced to 2 mm or less using a hammermill, pulverizer, and/or laboratory mill. To ensure sample homogeneity, the ground components should be thoroughly mixed. Finally, since samples may absorb moisture during the grinding and mixing processes, they should be redried for 4 hr at the previously specified temperature and then stored in a desiccator until the analyses are completed.

Sample Analysis

The nitrogen contents of standard and solid waste samples should be determined in triplicate by the following laboratory procedure. A reagent blank containing 2 g sucrose, previously dried at 105 C for 1 hr, should also be analyzed with each set of samples. In this manner any nitrogen present in the reagents will be detected, thus enabling the analyst to make appropriate corrections in the calculation of the nitrogen contents of the standard and solid waste samples.

Procedure	Comments
1. Weigh out approximately 2 g sucrose and transfer to a 800-ml Kjeldahl digestion flask labelled "Blank."	1. Glassine paper, used to support a sample during weighing and transferring, must not be added to the digestion flask.

2. Accurately weigh out three 1.0- to 2.5-g prepared solid waste samples and/or three standard samples (quantities previously specified) and transfer each to a labelled, 800-ml Kjeldahl digestion flask.	2. An analytical balance must be employed.
3. To each flask add 16 g potassium sulfate, 0.7 g mercuric oxide, 25 ml concentrated sulfuric acid, and a few plain Hengar granules.	3. A larger volume of acid will be required if the sample weight exceeds that recommended.
4. To the blank flask add three drops of silicone antifoaming agent.	
5. Place the flasks on the heating apparatus in an inclined position.	5. The carrier may be used to support the flasks during transfer.
6. Gently heat the contents of each flask until frothing ceases; boil briskly until the solution clears and for at least 30 min thereafter. (Samples containing organic material will require a 2- to 3-hr digestion.)	6. The heaters should be previously adjusted to bring 250 ml water at 25 C to a rolling boil in 5 min. Test heaters after preheating (10 min if gas, 30 min if electric). Use 3 or 4 boiling chips to prevent superheating of the water.
7. While the digested samples are cooling, align a 500-ml Erlenmeyer flask containing 50 ml boric acid under each condenser to be employed during the distillation of ammonia from the samples.	7. The tip of the condenser delivery tube must be beneath the surface of the boric acid solution to prevent the escape of ammonia.
8. To each cooled sample, add 200 ml distilled water and mix. Then add 0.5 g zinc and mix again.	
9. Pour 75 ml alkaline thiosulfate solution down the side of each flask and quickly attach a trap-condenser apparatus. Mix the contents of each flask and distill until the total volume of the contents of the receiving flask is 200 ml (50 ml boric acid solution plus 150 ml distillate).	9. a) CAUTION: The heaters should be turned on before connecting each flask to a trap-condenser apparatus. This will minimize the danger of liquid sucking back through the condenser. b) Immediately after mixing, lower the Erlenmeyer flask containing the boric acid solution so that the condenser delivery tube will drain and the pressure in the distillation flask will equalize.
10. Add 4 drops of the methyl purple solution to each Erlenmeyer flask and titrate the contents with the standard 0.1 N sulfuric acid solution to a light violet color. Record the ml of sulfuric acid employed for each sample.	10. The color of the solution will change from green to light gray and finally to light violet.
11. Calculate the percent nitrogen in each standard or solid waste sample as described in the following section.	

Calculations

The percent nitrogen in the samples should be calculated using the following formula:

$$\%\,\text{Nitrogen} = \frac{(A-B)\,(N)\,(14)\,(100)}{C}$$

where

A = ml 0.1 N sulfuric acid employed in the titration of the standard or solid waste sample
B = ml 0.1 N sulfuric acid employed in the titration of the blank
N = normality of the standard sulfuric acid solution
C = mg of standard or solid waste sample employed

Example:

$$\%\,\text{Nitrogen} = \frac{(12.61-0.30)\,(0.1040)\,(14)\,(100)}{1016} = 1.76$$

Accuracy and Precision

Analyses of acetanilide or disodium EDTA samples containing 50 mg or less of nitrogen always yielded 98.5 percent or more nitrogen recovery. If lower recoveries are observed, the analyst's technique should be suspected.

The reproducibility of this method has been determined by calculating the standard deviation of replicate determinations of the nitrogen contents of various standard and solid waste samples. The data are presented in Table 1.

TABLE 1
THE PRECISION OF THE METHOD

Type of sample and source	Lab No.	Number of replicate determinations per sample	Observed mean percent nitrogen	Standard deviation
Standards:				
Acetanilide		6	10.20	0.03
Disodium EDTA		11	7.41	0.07
Solid wastes:*				
Municipal refuse				
Cincinnati	1†	12	1.07	0.05
Johnson City	6	6	1.01	0.05
New York	RSL208‡	8	2.36	0.02
Compost				
Johnson City	W21B-DO	4	0.52	0.02
	W16B-D7	4	0.83	0.05
	W11B-D14	4	0.70	0.03
	W6B-D22	4	0.78	0.03
	W1B-D28	4	0.72	0.00
	W30A-D35	4	1.02	0.04
	W25A-D42	4	0.83	0.03
	X-D70	4	0.71	0.03
	X-FP	8	0.96	0.12
	W25D-SL-DL	6	0.88	0.02
Incinerator residue				
Cincinnati	RB-1	8	0.26	0.02
	RB-2	8	0.34	0.02
New York	RSL-205	6	0.75	0.04
	RSL-205-80M	3	0.87	0.01

*Glass and metals were removed from samples before analyses were initiated.

†The sample consisted primarily of dirt, leaves, and wood.

‡The sample consisted primarily of food wastes.

References

1. American Public Works Association. Municipal refuse disposal. Chicago, Public Administration Services, 1966. p. 390-391.
2. Gunning, J. W. Zietschrift für analytische Chemie, 28:188, 1889.
3. Horwitz, W., ed. Official methods of analysis of the Association of Agricultural Chemists. 10th ed. Section 2.044. Washington, D. C., Association of Agricultural Chemists, 1965, p. 16.
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THE COMPREHENSIVE NITROGEN METHOD

Discussion

In 1967 the comprehensive nitrogen method (CNM), a modification of the Kjeldahl method, was proposed by Gehrke et al. (1) for the determination of the nitrogen content of fertilizers, especially those containing high chloride-nitrate ratios. Subsequent investigations in our laboratory have demonstrated the applicability of the method in determining the total nitrogen (ammoniacal, organic, and nitrate) contents of municipal refuse, compost, and incinerator residue (2).

A sample of solid waste is first heated with metallic chromium in an acid medium to reduce the nitrates. The mixture is then digested with sulfuric acid. To increase the speed of the reaction and ensure the digestion of substances whose decomposition temperature is above the boiling point of sulfuric acid, mercuric oxide and potassium sulfate are added. After the digestion is completed, the mixture is treated with alkaline sodium thiosulfate, which destroys the mercuro-ammonium complex and permits the distillation of ammonia into 4-percent boric acid. A titration of the ammonium borate with standard sulfuric acid then enables the analyst to calculate the total (ammoniacal, organic, and nitrate) nitrogen content of the sample.

Safety Precautions

1. Safety glasses should always be worn by the analyst while handling concentrated sulfuric acid and digestion mixtures.
2. The analyst should exercise care in weighing, transferring, and disposing of mercuric oxide since inhalation, ingestion, or contact with the compound may cause mercurial poisoning.
3. Exhaust hoods or special traps should be employed during sample digestion to minimize the analyst's exposure to the evolving fumes.

Equipment

The equipment required for determining nitrogen in solid wastes by the comprehensive nitrogen-method is identical to that described in the preceding section on the Kjeldahl-Wilfarth-Gunning-Winkler method.

Reagents

The chemicals in the following list should all be reagent grade and nitrogen-free:

1. Sucrose
2. Acetanilide
3. Disodium ethylenediaminetetraacetic acid (disodium EDTA)
4. Potassium nitrate
5. Chromium metal, powder, 100 mesh
6. Hydrochloric acid, concentrated
7. Potassium sulfate
8. Mercuric oxide
9. Norton Alundum, 14 x

10. **Sulfuric acid, concentrated (95-98 percent)**
11. **Silicone antifoaming agent, General Electric No. 66 or Dow Corning Antifoam Q**
12. **Zinc metal, granulated**
13. **Alkaline thiosulfate solution: Dissolve 450 g sodium hydroxide in approximately 700 ml distilled water, cool, add 32 g sodium thiosulfate ($Na_2S_2O_3.5H_2O$), and dilute with distilled water to 1 liter. (The concentration of sodium thiosulfate has been decreased from 160 g/liter to 32 g/liter to permit the addition of a larger volume of the solution, or quantity of alkali, without increasing the quantity of thiosulfate used to precipitate the mercury.)**
14. **Boric acid solution: Dissolve 40 g of boric acid in distilled water and dilute with same to 1 liter.**
15. **Methyl purple solution (indicator): Dissolve 0.3125 g methyl red and 0.2062 g methylene blue in distilled water (or 0.1 percent ethyl alcohol) and dilute with same to 250 ml.**
16. **Sulfuric acid standard solution, 0.1 N: Dissolve approximately 3 ml concentrated sulfuric acid in 800 ml distilled water and dilute with same to 1 liter. Determine the normality of the solution, using a primary standard such as sodium borate.**

Technique Evaluation

Before beginning the characterization of solid waste samples, the investigator should evaluate his technique by employing the procedure in the analyses of compounds of known nitrogen content. A small quantity of an individual substance such as 0.25 g acetanilide, 0.5 g disodium EDTA, or 0.25 g potassium nitrate may be employed as a standard; but a mixture of either the aforementioned quantities of acetanilide and potassium nitrate or disodium EDTA and potassium nitrate is preferable. (See the section on accuracy and precision, p. 11.)

Solid Waste Sample Preparation

A solid waste sample should be prepared as described previously in the Kjeldahl-Wilfarth-Gunning-Winkler method.

Sample Analysis

The nitrogen contents of standard and solid waste samples should be determined in triplicate by the following laboratory procedure. A reagent blank, containing 2 g sucrose previously dried at 105 C for 1 hr, should also be analyzed with each set of samples. In this manner, any nitrogen present in the reagents will be detected, thus enabling the analyst to make appropriate corrections in the calculation of the nitrogen contents of the standard and solid waste samples.

Procedure	Comments
1. Weigh out approximately 2 g sucrose and transfer to a 800-ml Kjeldahl flask, labelled "Blank."	1. Glassine paper, employed to support a sample during weighing and transferring, must not be added to the digestion flask.
2. Accurately weigh out three 0.75- to 1.0-g, prepared solid waste samples and/or three standard samples (quantities previously	2. An analytical balance must be employed.

	specified) and transfer each to a labelled 800-ml Kjeldahl digestion flask.		
3.	To each flask add 1.2 g chromium and 35 ml distilled water. Let stand 10 min with occasional swirling.	3.	Swirling will ensure solution of all the nitrate present.
4.	Then add 7 ml concentrated hydrochloric acid to each flask. Let stand until a visible reaction occurs.	4.	a) Add a few drops of antifoaming reagent if foaming is anticipated. b) Allow 1 to 5 min for reaction.
5.	Transfer each flask to a burner and heat its contents to a rolling boil (maximum heating period is 5 min). Remove from the burner and cool.	5.	a) The carrier should be used to transport the flasks. b) The burners should be previously adjusted to bring 250 ml water at 25 C to a rolling boil in 5 to 7.5 min. Test heaters after preheating (10 min if gas, or 30 min if electric). Use 3 or 4 boiling chips to prevent superheating of the water.
6.	Add 22 g potassium sulfate, 1.0 g mercuric oxide, and 1.5 g alundum to each sample.		
7.	Transfer the flasks to the hood and add 25 ml concentrated sulfuric acid to each.	7.	Additional sulfuric acid will be required if the quantity of sample exceeds that recommended.
8.	Add 3 drops of the antifoaming reagent to the blank and any other sample containing considerable organic material.		
9.	Place each flask on a preheated burner adjusted to give a 5- to 7.5-min boil test, and digest the sample for 1 to 1 1/2 hr with occasional gentle swirling.	9.	a) See the burner adjustment described in comment 5 b. b) The use of a preheated burner minimizes foaming at this stage. c) It should take 15 to 20 min for the copious white fumes to clear out of the bulb of the flask. Most samples should then be digested for an additional 30 min. If organic material containing refractory nitrogen is present, digest for a total of 60 min after the white fumes have evolved from the flask.
10.	While the digested samples are cooling, align a 500-ml Erlenmeyer flask containing 50 ml boric acid under each condenser to be employed during the distillation of ammonia from the samples.	10.	The tip of the condenser delivery tube must be beneath the surface of the boric acid solution to prevent the escape of ammonia.
11.	To each cooled sample, add 200 ml distilled water and mix. Then add 0.5 g zinc and mix again.		
12.	Pour 125 ml alkaline thiosulfate solution down the side of the flask and quickly attach a trap-condenser apparatus. Mix	12.	a) The heaters should be turned on before connecting each flask to a trap-condenser apparatus. This will minimize the

the contents of each flask and distill until the total volume of the contents of the receiving flask is 200 ml (50 ml boric acid plus 150 ml distillate).

13. Add 4 drops of methyl purple to each Erlenmeyer flask and titrate the contents with the standard 0.1 N sulfuric acid solution to a light violet color. Record the milliliters of sulfuric acid employed for each sample.
14. Calculate the percent nitrogen in each standard or solid waste sample as described in the following section.

danger of liquid sucking back through the condenser.

b) Immediately after mixing, lower the Erlenmeyer flask containing the boric acid solution so that the condenser delivery tube will drain and the pressure in the distillation flask will equalize.

13. The color of the solution will change from green to light gray, and finally to light violet.

Calculations

The percent nitrogen in the samples should be calculated using the following formula:

$$\%\,\text{Nitrogen} = \frac{(A-B)\,(N)\,(14)\,(100)}{C}$$

where

A = ml 0.1 N sulfuric acid employed in the titration of the standard or solid waste sample
B = ml 0.1 N sulfuric acid employed in the titration of the blank
N = normality of the standard sulfuric acid solution
C = mg of standard or solid waste sample employed

Example.

$$\%\,\text{Nitrogen} = \frac{(12.61-0.30)\,(0.1040)\,(14)\,(100)}{1016} = 1.76$$

Accuracy and Precision

Analyses of acetanilide, disodium EDTA, and potassium nitrate samples containing less than 60 mg nitrogen always yielded 98.2 percent or more nitrogen recovery. If lower recoveries are observed, the analyst's technique should be suspected.

The reproducibility of the method has been determined by calculating the standard deviation of replicate determinations of the nitrogen contents of various standard and solid waste samples. The data are presented in Table 1.

TABLE 1
PRECISION OF THE METHOD

Type of sample and source	Lab No.	Number of replicate determinations per sample	Observed mean percent nitrogen	Standard deviation
Standards:				
Acetanilide		6	10.27	0.04
Disodium EDTA		11	7.39	0.09
Potassium nitrate		11	13.64	0.09
Solid wastes:*				
Municipal refuse				
Cincinnati	5†	8	0.32	0.01
Johnson City	6	6	0.94	0.04
New York	RSL-208‡	10	2.37	0.04
Memphis	RSL-294	8	0.87	0.04
Ogden	RSL-12	6	0.53	0.04
Compost				
Johnson City	W25D-SL-D1	6	0.93	0.01
St. Petersburg	SPF	6	1.03	0.05
	SPF-Fort.	6	8.02	0.14
Jamaica	JAM	6	0.52	0.04
Incinerator residue:				
Cincinnati	RB-2	6	0.32	0.02
Memphis	RSL-292C	6	2.49	0.02
	RSL-292I	6	0.04	0.01
Ogden	RSL-17	5	0.08	0.01
New York	RSL-205	8	0.74	0.03

*Glass and metals were removed from samples before analyses were initiated.

†This was a simulated sample.

‡This sample consisted primarily of food wastes.

References

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THE AUTOMATED DUMAS METHOD

Discussion

In 1960, G.M. Gustin described a simple automatic apparatus for the rapid determination of nitrogen by the Dumas procedure (1). Shortly thereafter, the Coleman Instrument Corporation manufactured an instrument capable of the automatic analysis of grain, fertilizers, soils, meat products, feeds, biological materials, and other substances dissociable at temperatures under 1,100 C. Investigators in our laboratory have demonstrated that the Coleman Nitrogen Analyzer II, Model 29A, (see Figure 1) may also be employed successfully in the characterization of municipal refuse, compost, and incinerator residue (2).

A 100-mg (or less), dry, homogeneous solid waste sample is first packed in a combustion tube. After the tube has been inserted in the combustion train, high purity carbon dioxide is employed to purge the system of entrapped air. The sample is then decomposed at 850 to 900 C in the presence of oxidizing agents such as Cuprox (copper oxide) and Coboxide (cobalt oxide). The gaseous combustion products (nitrogen, nitrogen oxides, methane, carbon monoxide, and carbon dioxide) are swept along through the system by the flow of high-purity carbon dioxide. A platinum catalyst mixed with the Cuprox packed in the combustion and post-heater tubes ensures the complete combustion or oxidation of methane and carbon monoxide. The reduction of the nitrogen oxides to gaseous nitrogen is effected by the Cuprin (reduced copper) in the post-heater tube. The gaseous mixture flowing from the latter tube is then scrubbed thoroughly in a caustic solution to remove all its carbon dioxide content. The remaining nitrogen is collected and measured in a 50,000-μl stainless steel syringe linked to a digital counter. Since the purging, combusting, and sweeping actions are entirely automatic, a sample analysis can be performed with ease in 15 min.

Safety Precautions

1. The analyst should wear cotton gloves while handling the cold combustion or post-heater tubes. (Observations in our laboratory have indicated that the tube durability, and hence procedural safety, may be decreased by repeated alternate exposures of the tube to perspiring hands and furnace temperature.)
2. During the analysis of substances that pyrolyze rapidly or detonate, a protective shield should be attached to the side of the instrument and positioned in front of the combustion tube. If a shield is temporarily unavailable, safety glasses should be worn to protect the eyes.
3. The temperature of the combustion tube furnaces should always be maintained below 1020 C to prevent the sintering of the Cuprox tube packing and the possible explosion of the combustion tube.
4. The analyst should always remove a hot combustion tube from the train by carefully grasping the cooler ends of the tube. NEVER grasp the hot center of the tube.
5. The analyst should wear an asbestos glove when inserting his hand into the muffle furnace to mix or remove the Cuprox – platinum catalyst reagent being regenerated at 800 C.

Equipment

Requirements.

1. Analyzer, Nitrogen II, Coleman Model 29A, complete with nitrometer (Coleman #29-312); three Vycor combustion tubes (Coleman #29-328); Vycor post-heater tube (Coleman #29-337);

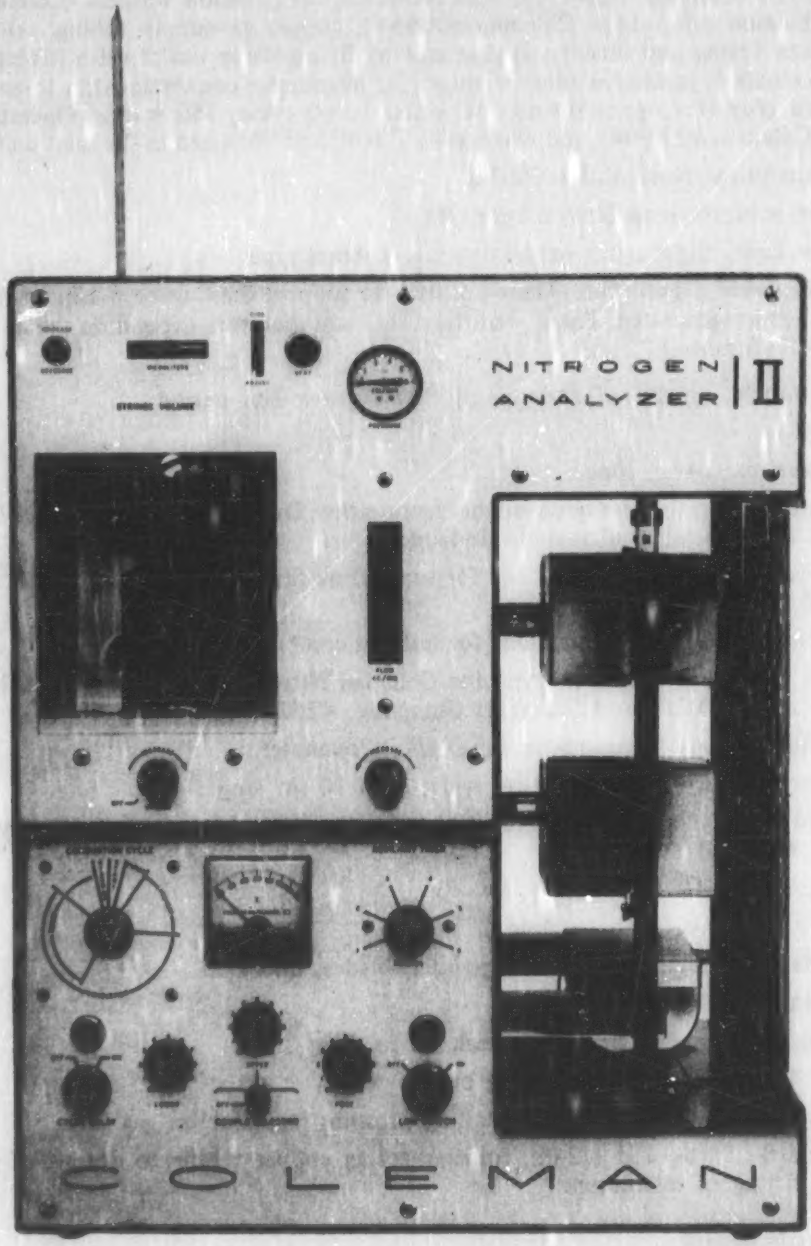
NITROGEN
ANALYZER II
COLEMAN

Figure 1. Coleman Nitrogen Analyzer II, Model 29A. (Courtesy of Coleman Instruments Division of the Perkin-Elmer Corporation.)

special scale thermometer (Coleman #29-340); 1 package of 100 disposable aluminum combustion boats (Coleman #29-412); four Neoprene compression washers (Coleman #9584-V); two combustion tube plugs (Coleman #9595V); copper gas-supply tubing, 60 in. long, with compression fitting and bushing at one end to fit regulator outlet valve (Coleman #3825V); two spring-loaded, joint-type pinch clamps (for nitrometer connections); a three-wire cord and three-blade plug (for operation on 115 volts, 50/60 cycle, 750 watts); Operating Directions D360-C (Coleman #29-904); and reagents 4, 5, 6, 8, and 10, listed in the next section

2. Balance, analytical, readability 0.0001-g
3. Barometer, accurate, readability 0.5-mm Hg
4. Desiccator, large, either Pyrex or stainless steel cabinet type
5. Dishes, evaporating, porcelain (These are used to support the Cuprox platinum catalyst reagent while it is being regenerated. The size of the dishes will therefore depend on the inner dimensions of the muffle furnace.)
6. Furnace, muffle, capable of maintaining 800 C for a 2-hr period
7. Glass wool
8. Gloves, asbestos, 14 in. long
9. Gloves, cotton, large (These are available from either Davis Gloves, Springfield, Ohio, or Wash Rite, Inc., 1410 Cornell, Indianapolis, Indiana.)
10. Mats, asbestos, 1/8 by 12 by 12 in., for supporting the hot evaporating dishes after they are removed from the furnace.
11. Rack, aluminum (or cadmium-plated) for holding combustion tubes
12. Regulator, two stage, Coleman type for Coleman Nitrogen Analyzer, with CGA connection #320 (Model 8C, Mathieson Scientific Company, #26285-05)
13. Rod, stirring, teflon-coated, 15 in. long, 1/4 in. diameter
14. Screwdriver, slot drive, blade 1/4 in. wide, 6 to 10 in. long
15. Shield, protective, for use in combustion of substances that pyrolyze rapidly or detonate (Coleman #33-450)
16. Sieve, brass, 8-in., US #40
17. Sieve cover and receiver, brass, 8-in.
18. Spatula, stainless steel, rounded and pointed blades, micro
19. Support, gas cylinder
20. Tongs, crucible type, nickel plated steel, 18 in. long
21. Tweezers, 4-1/2 in. long, for grasping boats
22. Wrench, open end, 1 1/8 in., for installing pressure regulator on gas tank
23. Wrenches, 3/8-, 7/16-, and 1/2-in., for connecting copper tubing to output of gas regulator and input fitting of instrument

Assembly and maintenance.

The Coleman Nitrogen Analyzer and its accessories should be assembled, evaluated, and maintained as described in the operating directions for the instrument.

Reagents

Requirements.

1. Acetanilide, ACS reagent grade, crystalline
2. Carbon dioxide, Coleman instrument grade, 99.99 percent pure (cylinder size 1)
3. Causticon, potassium hydroxide solution, 1 pint (Coleman #29-110)
4. Coboxide, cobalt oxide, 30-g bottle (Coleman #29-170)
5. Cuprin, metallic copper, 1-lb bottle (Coleman #29-120)
6. Cuprox, copper oxide, fines, 1-lb bottle (Coleman #29-140)
7. Cuprox – platinum catalyst reagent, 5-lb bottle (Coleman #29-165)
8. Disodium ethylenediaminetetraacetic acid, ACS reagent grade, powder (Minimum assay: 99 percent)
9. Mercury, reagent, redistilled, 50 ml
10. Potassium nitrate, ACS, reagent grade
11. Salicylic acid, ACS, reagent grade, nitrogen free
12. Silica gel, indicating, mesh size 6-16, 5 lb unit
13. Silver vandanate, 40-g bottle (Coleman #33-130)

Preparation, maintenance, and storage.

All fresh reagents should be employed as received. The replacement, regeneration, and/or maintenance of the reagents should be accomplished as outlined in the operating directions for the instrument. All capped (but unsealed) reagent bottles containing solids should be stored in a desiccator to minimize the sorption of moisture.

Technique Evaluation

Before initiating the analysis of solid waste samples, the investigator should evaluate his technique by employing the procedure in the analyses of compounds of known nitrogen content. Either 0.05 g acetanilide or 0.10 g disodium EDTA should be used routinely as a standard sample. To evaluate the recovery of nitrate nitrogen, the analyst should use a 0.1-g potassium nitrate sample to which an equal weight (i.e., 0.1 g) of nitrogen-free salicylic acid has been added. (See section on accuracy and precision.)

Solid Waste Sample Preparation

The procedure for the preparation of a solid waste sample is the same as that previously outlined in the Kjeldahl-Wilfarth-Gunning-Winkler method, except that the sample's particle size should be reduced to 0.5 mm or less before the mixing is begun.

Sample Analysis

The analyst should employ the following instructions in conjunction with the sample analysis procedures outlined in the operating directions for the instrument:

1. All determinations (including blanks) should be performed in triplicate.
2. The sample should contain 100 mg (or less) organic matter and 40 mg (or less) nitrogen.
3. The combustion tube packing, employed in all analyses, should be modified by adding Cuprox fines 1/4 in. below, 1/4 in. above, and completely around the aluminum boat. This packing, if carefully performed, will prevent the fusion of the boat with the Vycor combustion tube.
4. The instrument should be operated on the normal 12-min. cycle.
5. The furnace controls should be adjusted so that the post-heater tube furnace is maintained at 700 C and the lower and upper combustion tube furnaces attain 850 to 900 C during the final portion of the combustion period. (Occasionally, as in the analysis of the potassium nitrate-salicylic acid standard mixture, the analyst may observe a sintering of the Cuprox around the sample in the combustion tube. Lower combustion furnace settings should then be employed to minimize hazards and increase tube life.)
6. Daily furnace warm-up periods may be avoided by leaving the instrument in the standby position overnight. (The nitrometer must be disconnected as usual.)

Calculations

The percent nitrogen in a standard or solid waste sample should be determined as follows:

1. Record V_O, the observed volume of nitrogen (μl).

$$V_O = R_2 - R_1$$

where

R_1 = the initial counter reading
R_2 = the final counter reading

2. Determine V_c, the corrected nitrogen volume (μl)

$$V_c = V_o - (V_b + V_t)$$

where

V_b = volume of blank (μl)
V_t = volume correction for temperature (μl)

$$= C_f\,(t_2 - t_1)$$

where

C_f = correction factor per degree Kelvin (Table 1)
t_1 = the initial syringe temperature in degrees Kelvin
t_2 = the final syringe temperature in degrees Kelvin

3. Determine P_c, the corrected barometric pressure (mm Hg)

$$P_c = P_o - (P_b + P_v)$$

where

P_o = the observed barometric pressure (mm Hg)
P_b = the pressure correction for temperature (Table 2)
P_v = the pressure correction for the vapor pressure of potassium hydroxide (Table 3)

TABLE 1
CORRECTION FACTOR (C_f) EMPLOYED IN THE CALCULATION OF THE VOLUME CORRECTION FOR TEMPERATURE (V_t)*†

Final counter reading (µl)	C_f‡ (µl/K)	Final counter reading (µl)	C_f‡ (µl/K)
0	12	24,000	92
1,000	15	25,000	95
2,000	19	26,000	98
3,000	22	27,000	102
4,000	26	28,000	105
5,000	29	29,000	109
6,000	32	30,000	112
7,000	35	31,000	115
8,000	39	32,000	119
9,000	42	33,000	122
10,000	45	34,000	127
11,000	48	35,000	129
12,000	52	36,000	132
13,000	55	37,000	135
14,000	59	38,000	139
15,000	62	39,000	142
16,000	65	40,000	145
17,000	69	41,000	148
18,000	72	42,000	152
19,000	76	43,000	155
20,000	79	44,000	159
21,000	82	45,000	162
22,000	85	46,000	165
23,000	89	47,000	169

*Based on Coleman Instrument Corporation, D-360C Operating Directions for the Coleman Model 29A Nitrogen Analyzer II, Maywood, Ill., 1966. p. 22.

†$V_t = C_f\,(t_2 - t_1)$ where t_1 and t_2 are the initial and final temperatures, expressed in degrees Kelvin, respectively.

‡Factor applicable only for measurements made with nitrometers having check valves.

TABLE 2
PRESSURE CORRECTION (P_b) FOR TEMPERATURE*

Room Temperature (C)	P_b (mm Hg)	
	P_o† = 700 to 749	P_o = 750 to 780
10	1.2	1.3
15	1.8	1.9
20	2.3	2.5
21	2.4	2.6
22	2.5	2.7
23	2.7	2.9
24	2.8	3.0
25	2.9	3.1
26	3.0	3.2
27	3.1	3.3
28	3.3	3.5
29	3.4	3.6
30	3.5	3.7
31	3.6	3.8
32	3.7	3.9
33	3.9	4.1
34	4.0	4.2
35	4.1	4.3

*Based on Coleman Instrument Corporation, D-360C Operating Directions for the Coleman Model 29A Nitrogen Analyzer II, Maywood, Ill. 1966, p. 22.

†P_o = observed barometric pressure in mm Hg.

TABLE 3
PRESSURE CORRECTION (P_v) FOR VAPOR PRESSURE OF POTASSIUM HYDROXIDE*

Temperature† (K)	P_v (mm Hg)
288	4.1
293	5.7
298	7.4
299	7.8
300	8.3
301	8.7
302	9.2
303	9.6
304	10.2
305	10.8
306	11.3
307	11.9
308	12.5
309	13.3
310	14.1
311	14.9
312	15.7
313	16.5

*Based on Coleman Instrument Corporation, D-360C Operating Directions for the Coleman Model 29A Nitrogen Analyzer II, Maywood, Ill., 1966, p. 22.

†For practical purposes, the temperature of the potassium hydroxide is the same as that of the syringe.

4. Determine percent nitrogen, using the following formula:

$$\%N = \frac{P_c}{T} \times \frac{V_c}{W} \times 0.0449$$

where

T = t_2, the final syringe temperature in degrees Kelvin
W = the sample weight in mg

Example:

$$\begin{aligned}
\text{If } P_o &= 747.2 \text{ mm Hg at } 24.9 \text{ C} \\
W &= 39.7 \\
R_1 \text{ (blank)} &= 5{,}219 \\
R_2 \text{ (blank)} &= 5{,}270 \\
R_1 \text{ (solid waste)} &= 5{,}270 \\
R_2 \text{ (solid waste)} &= 5{,}620 \\
t_1 &= 303.1 \\
t_2 \text{ (or T)} &= 303.3 \\
\text{Then } V_b \text{ (blank)} &= 51 \\
V_o \text{ (solid waste)} &= 350 \\
V_c \text{ (solid waste)} &= 350 - [51 + (29)(303.3 - 303.1)] \\
&= 293.2 \\
P_c &= 747.2 - (2.9 + 9.6) \\
&= 734.7 \\
\%N &= \frac{734.7}{303.3} \times \frac{293.2}{39.7} \times 0.0449 \\
&= 0.80
\end{aligned}$$

Accuracy and Precision

Analyses of acetanilide, disodium EDTA, or potassium nitrate samples containing less than 20 mg nitrogen always yielded 99 percent or more nitrogen recovery. If lower recoveries are observed, the analyst's technique should be suspected.

The reproducibility of the method has been determined by calculating the standard deviation of replicate determinations of various standards and solid waste samples. The data are presented in Table 4.

TABLE 4
THE PRECISION OF THE METHOD

Type of sample and source	Lab No.	Number of replicate determinations per sample	Observed mean percent nitrogen	Standard deviation
Standards:				
Acetanilide		10	10.34	0.06
Disodium EDTA		14	7.56	0.02
Potassium nitrate		6	13.72	0.06
Solid wastes:*				
Municipal refuse				
Cincinnati	5†	6	0.25	0.06
Johnson City	6	6	0.92	0.03
New York	RSL-208‡	6	2.44	0.06
Memphis	RSL-294	6	0.72	0.07
Ogden	RSL-12	10	0.49	0.06
Compost				
Johnson City	W11B-D14	6	0.78	0.08
	W25D-SL-D1	12	0.82	0.06
St. Petersburg	SPF	6	1.09	0.03
	SPF-Fort.	10	8.16	0.10
Jamaica	JAM	9	0.57	0.05
Incinerator residue				
Cincinnati	RB-2	5	0.28	0.03
Memphis	RSL-292C	6	2.55	0.06
	RSL-292I	6	0.03	0.01
Ogden	RSL-17	6	0.08	0.01
New York	RSL-205	3	0.84	0.00

*Glass and metals were removed from samples before analyses were initiated.

†This was a simulated sample.

‡This sample consisted primarily of food wastes.

References

1. Gustin, G.M. A simple, rapid automatic micro-Dumas apparatus for nitrogen determination. Microchemical Journal, 4:43-54, 1960.
2. Ulmer, N.S., and W.H. Kaylor. An evaluation of the applicability of three methods for the determination of nitrogen in solid wastes. Cincinnati, Solid Waste Research Laboratory departmental report, 1971.

APPENDIX

A Brief History of the Kjeldahl Method

The historical background and evolution of the Kjeldahl method have been thoroughly reviewed by R.B. Bradstreet (1). The following brief resume will hopefully afford the analyst a greater appreciation of the ingenuity and resourcefulness of the many scientists who contributed to the development of the method.

In 1883 Johann Kjeldahl, a Danish chemist associated with the Carlsberg Laboratory in Copenhagen, published a new method for the determination of nitrogen. The procedure, developed primarily to facilitate a study of the protein changes in grain, consisted of the following steps: (a) Heating a sample for 2 hr in concentrated sulfuric acid to which fuming sulfuric acid and phosphoric anhydride had been added, (b) subsequent oxidation with powdered permanganate, (c) dilution and transfer of the mixture to a distillation flask, (d) addition of alkali and then zinc, (e) distillation of the ammonia into standard acid, (f) addition of potassium iodide and iodate to the distillate, and finally (g) a titration of the liberated iodine with standard thiosulfate.

Shortly after the publication of this method, Kjeldahl's contemporaries proposed numerous modifications and improvements. In 1885, Wilfarth reported that the speed of the digestion could be accelerated by the addition of a copper salt. He also described the catalytic effects of the oxides of copper, iron, mercury, bismuth, manganese, zinc, and lead. Since mercuric oxide, the most effective catalyst, tended to form a complex with ammonia and thus lower nitrogen recovery, its use was discontinued until chemists discovered that the complex could be destroyed by the addition of alkaline sulfide, thiosulfate, monosodium phosphate, potassium xanthanate, or potassium arsenate. In the years following Wilfarth's significant contribution, investigators have elucidated and established many other substances capable of accelerating specific digestion reactions. The addition of a catalyst has therefore become a routine and universally accepted step in the Kjeldahl procedure.

In 1889, J.W. Gunning reported that the addition of potassium sulfate to the digestion mixture would raise its boiling point, increase the severity of the reaction, shorten the digestion period, and hence permit the analyses of other types and sizes of samples. Although the applicability of many other salts has been evaluated since Gunning's observations were published, potassium sulfate is still recommended, particularly for the analysis of refractory compounds. The lower acid-salt ratios attainable with this salt have permitted digestion at higher temperatures without loss of nitrogen.

Although the suggestions of Wilfarth and Gunning greatly increased the scope of the Kjeldahl method, research has since demonstrated that nitro, nitroso, azo, aminoazo, hydrazine, and other compounds in which a nitrogen atom is linked to an oxygen atom (or atoms) or to a second nitrogen atom must be reduced before their nitrogen contents can be recovered quantitatively. This reduction has been accomplished in two general ways: (a) as a pretreatment of the sample with substances such as powdered copper, zinc, chromium, titanous chloride, potassium iodide, and sodium hydrosulfite, and (b) by the addition of compounds such as sucrose, benzoic acid, phenol, and salicylic acid directly to the acid and sample. The latter compounds supply a reducing effect during the Kjeldahl digestion as they decompose to free carbon with subsequent reduction of the sulfuric acid to sulfur dioxide.

Kjeldahl originally proposed that powdered potassium permanganate be added to the sample upon completion of the acid-digestion. Although he believed this step necessary to ensure the complete oxidation or conversion of any nitrogen not previously converted to ammonium sulfate by the digestion, it was later discontinued when variations in nitrogen recovery were observed. During the

20th century, other oxidizing agents such as hydrogen peroxide, perchloric acid, and potassium persulfate have occasionally been employed with more favorable results.

The procedure proposed by Kjeldahl for the recovery of ammonia and the final determination of nitrogen has also been modified by numerous investigators. If the ammonia is distilled, a direct heat distillation is usually employed; aeration and steam distillation techniques have been proposed, however. Kjeldahl's time-consuming titration has been replaced either by a back titration with a known volume of standard acid or the direct titration of ammonium borate, as proposed by Winkler in 1913. Other methods such as gravimetry, nesslerization, pH measurement, colorimetric reaction, or neutralization of the digest followed by direct estimation of the ammonia have also been utilized successfully in the analysis of samples.

References

1. Bradstreet, R.B. The Kjeldahl method for organic nitrogen. New York, Academic Press, 1965. 238 p.

LABORATORY PROCEDURE FOR THE GRAVIMETRIC DETERMINATION OF CARBONATE CARBON IN SOLID WASTES

Donald L. Wilson*

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DISCUSSION

Inorganic carbon measurements on solid waste samples, in conjunction with total carbon measurements, (1) serve as a criteria for evaluating incinerator efficiency to dispose of organic carbon matter. High carbonate content in incinerator residue will cause hardness in leachate water.

The carbonate analysis is also necessary because the carbonate content affects the analysis of other constituents and the analytical interpretation of other methods. For example, the precision and accuracy of the ash-volatile determination (2) (loss-on-ignition or L.O.I. at 600 C) can vary with the degree of decomposition of carbonates present (3). Our laboratory investigations showed that during the calorific determination, the degree of decomposition of calcium carbonate in incinerator residue samples depends upon the amount of combustion aid added to the sample (4). The carbonate content of samples must be known in order to calculate their calorific values from ultimate analyses. The oxygen content of samples is normally determined by subtracting from the volatile portion of a sample the carbon, hydrogen, nitrogen, and sulfur contents. Such oxygen values may be in great error because of not considering the oxygen combined with the carbonates and metal oxides in the residue left from the volatile analysis (5).

An existing AOAC method (6) for determining carbonate carbon was found feasible for solid waste samples. (7) Such samples with carbonate carbon contents from 0.05 to 8.00 percent have been precisely and accurately analyzed.

Before the carbonate carbon content is determined, all solid waste samples must be dried, ground to less than 2 mm, and thoroughly mixed. Employing between 1 to 5 g of sample in each determination produces data that are precise and accurate to a satisfactory degree.

Carbonate carbon is determined gravimetrically after (1) reacting a weighed, dry, uniform sample with dilute hydrochloric acid inside a closed system, and (2) fixing the evolved gases in an absorption train (Figure 1). The procedure (Figure 2) is designed to measure the total carbonate carbon in dry solid waste samples.

APPARATUS

Requirements

1. Absorption bulb, Nesbitt, Pyrex; two or more (Fisher #7-517)
2. Balance, analytical, 200-g capacity, 0.1-mg readability (Sartorius, 2400 Series)
3. Basket, test tube (Fisher #14-966D)
4. Beaker, Griffin low form, 250-ml
5. Bottle, gas-washing, Pyrex, with fritted cylinder, coarse porosity, 250-ml capacity; two
6. Bottle, narrow mouth, standard taper, 2,000 ml
7. Burner, gas
8. Clamp, utility, vinylized jaws, three-prong grip; five
9. Condenser, Kimble Modern Liebig, 400-mm jacket (Fisher #7-704 C)

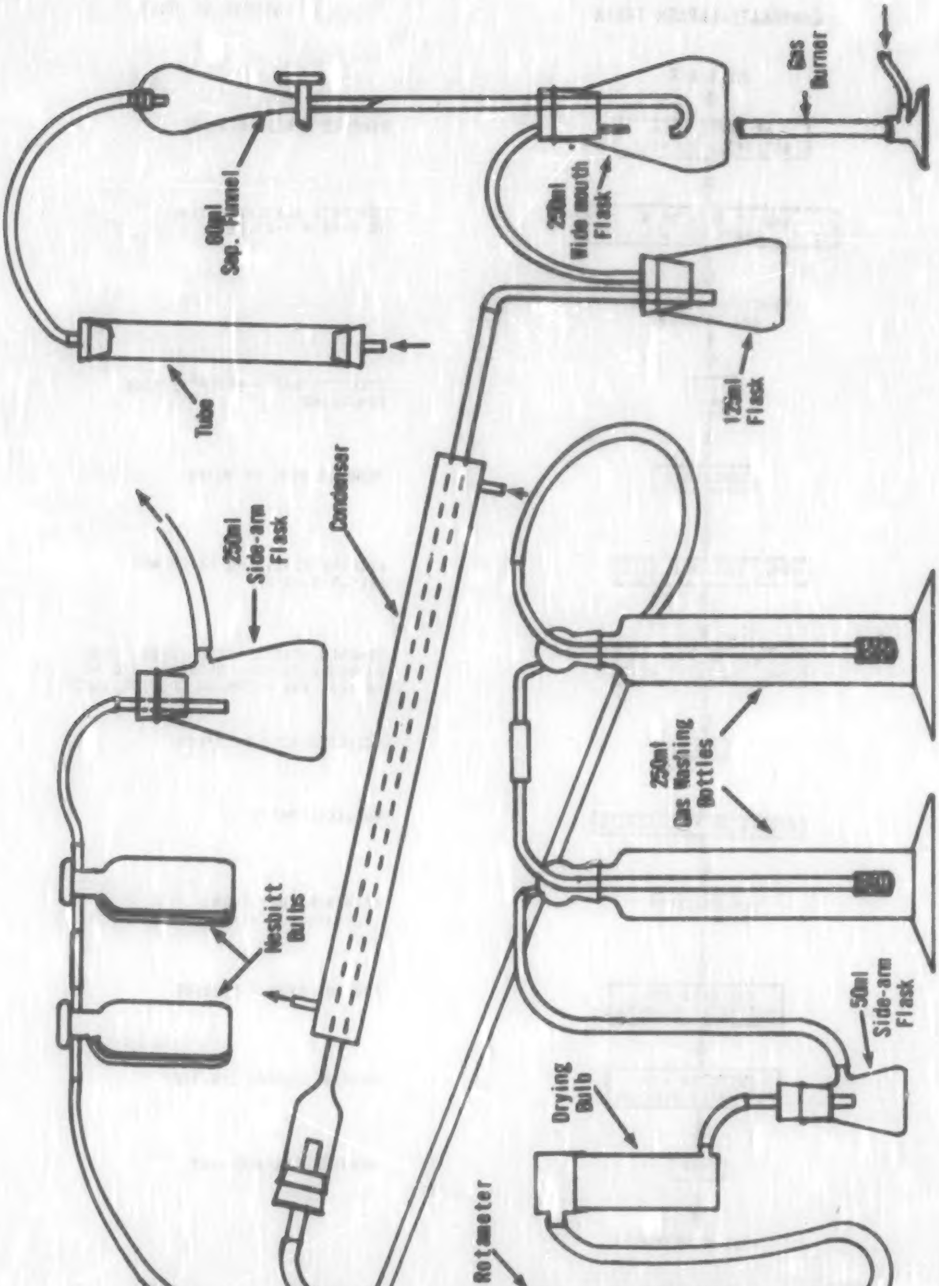
Gas Burner
250ml Wide mouth Flask
60ml Sep. Funnel
Tube
125ml Flask
Condenser
250ml Side-arm Flask
250ml Gas Washing Bottles
Nesbitt Bulbs
50ml Side-arm Flask
Drying Bulb
Rotameter

Figure 1. General schematic of carbonate-carbon train.

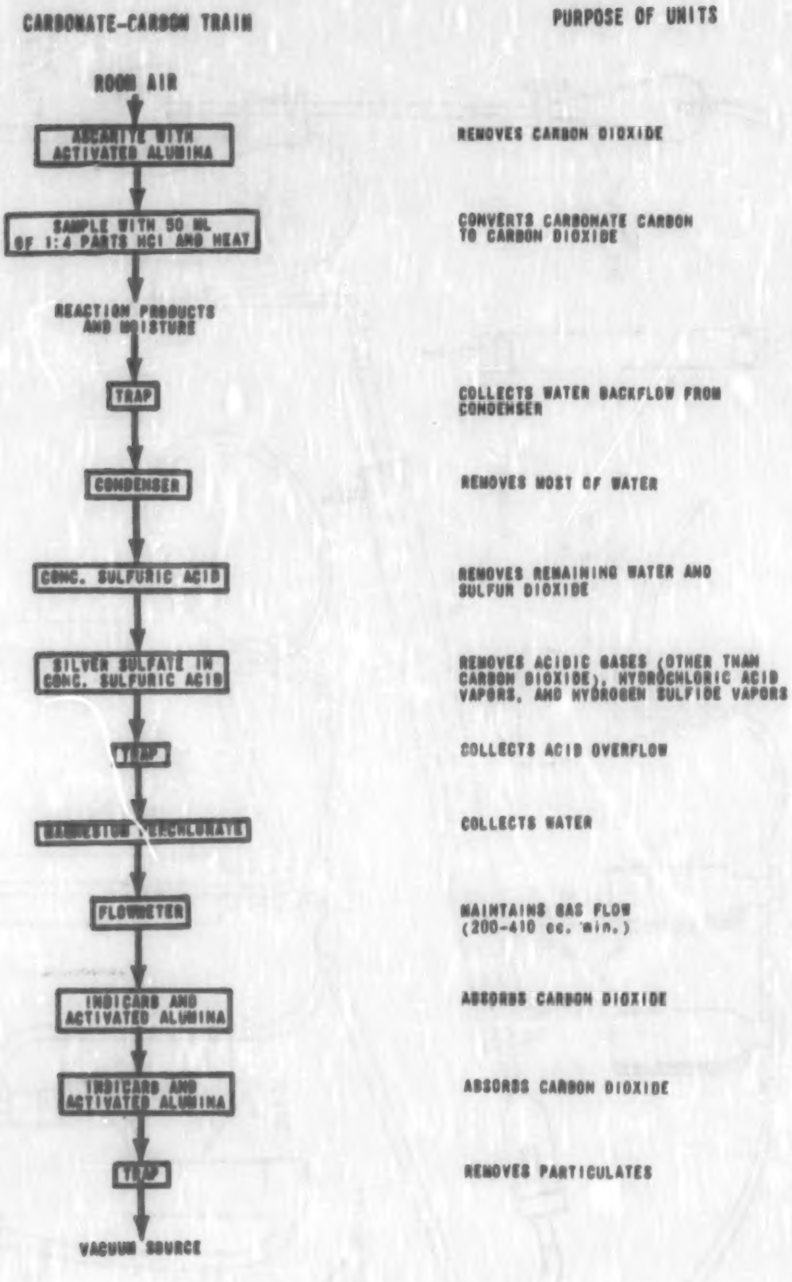
CARBONATE-CARBON TRAIN
PURPOSE OF UNITS
ROOM AIR
ASCARITE WITH ACTIVATED ALUMINA
REMOVES CARBON DIOXIDE
SAMPLE WITH 50 ML OF 1:4 PARTS HCl AND HEAT
CONVERTS CARBONATE CARBON TO CARBON DIOXIDE
REACTION PRODUCTS AND MOISTURE
TRAP
COLLECTS WATER BACKFLOW FROM CONDENSER
CONDENSER
REMOVES MOST OF WATER
CONC. SULFURIC ACID
REMOVES REMAINING WATER AND SULFUR DIOXIDE
SILVER SULFATE IN CONC. SULFURIC ACID
REMOVES ACIDIC GASES (OTHER THAN CARBON DIOXIDE), HYDROCHLORIC ACID VAPORS, AND HYDROGEN SULFIDE VAPORS
TRAP
COLLECTS ACID OVERFLOW
MAGNESIUM PERCHLORATE
COLLECTS WATER
FLOWMETER
MAINTAINS GAS FLOW (200-410 cc. min.)
INDICARB AND ACTIVATED ALUMINA
ABSORBS CARBON DIOXIDE
INDICARB AND ACTIVATED ALUMINA
ABSORBS CARBON DIOXIDE
TRAP
REMOVES PARTICULATES
VACUUM SOURCE

Figure 2. General outline of carbonate-carbon train.

10. Cylinder, graduated, 50-ml
11. Cylinder, graduated, 100-ml
12. Cylinder, graduated, 500-ml
13. Drying bulb, unique type (absorption bulb may be used)
14. Erlenmeyer flask, wide-mouth, 125-ml
15. Erlenmeyer flask, wide mouth, 250 ml; four or more
16. Flask, filtering, side-arm, 50-ml
17. Flask, filtering, side-arm, 250-ml
18. Funnel, separatory, 60-ml
19. Gloves, lint-free
20. Hot plate (Fisher #11-494 or equivalent)
21. Rod, glass, about 6 in. long
22. Rotameter or flowrator meter, size 02, sapphire float, hose connections, tri-float tube #02F-1/8-16-5 (Fischer and Porter #10A-1017 or equivalent)
23. Ring, support, with clamp, 4-in. O.D.
24. Stopcock, hard rubber (Fisher #14-630) (optional)
25. Stopper, one-hole, size 0
26. Stopper, one-hole, size 5; two
27. Stopper, one-hole, size 6; three
28. Stopper, two-hole, size 6; two
29. Stopper, two-hole, size 9
30. Support stand, rectangular base, 24-in. rod; two
31. Support stand, rectangular base, 36-in. rod
32. Timer (Fisher #6-662)
33. Tube, glass, about 1-in. diameter, about 6 in. long
34. Tubing, bubble, Argyle universal, plain, lumen, 3/16-in. bore (Aloe Scientific #AR-500)
35. Tubing, glass, 3/16-in. bore, about 2 ft
36. Tubing, rubber, red, thin-wall, 3/16-in. bore (Fisher #14-166)
37. Tubing, Tygon, 1/4-in. I.D., 3/8-in. O.D., 1/16-in. wall
38. Tubing, vacuum-pressure, 3/8-in. I.D.
39. Vacuum source and regulator valve or clamp
40. Wire square, asbestos center, 5-in sq
41. Alkalimeter, Knoor, carbon dioxide apparatus (Fisher #1-198)
42. Flask, Erlenmeyer form, with standard taper joint-24/40, Pyrex, 250-ml. (Fisher #10-047C) (a corequisite of item 41)*

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*If items 41 and 42 are purchased, then items 9, 15, 18, 25, 29, 33, and two of item 27 are unnecessary.

Preparation

Assembling the train.

The components of the apparatus are assembled in the following sequence:

a. Glass tube: Activated alumina is placed in the lower fourth and ascarite topped with activated alumina in the upper part. A small layer of glass wool is inserted beneath the lower activated alumina and above the upper activated alumina. The glass tube is closed at each end with a one-hole, size 6 stopper. About 1 in. of glass tubing protrudes from the hole in the stopper. The room air-flow into the tube is through the bottom inlet. The glass tube is attached to a 36-in.-rod support stand with a utility clamp.

b. Separatory funnel (60-ml, with one-hole, size 0 stopper) is attached to 36-in.-rod support stand with utility clamp.

c. Erlenmeyer flask, 250-ml, with two-hole, size 9 stopper and glass tubing inserted through stopper holes. Bend one piece of glass tubing upward at the end just enough to keep it from hitting the bottom of the flask; rest the flask on wire square on ring support, which is attached to the 36-in.-rod support stand.

d. Set gas burner under Erlenmeyer flask (item c).

e. Erlenmeyer flask, 125-ml, with two-hole, size 6 stopper and glass tubing inserted through stopper. This item serves as a liquid trap. If an alkalimeter is employed, the flask is placed after the condenser of the alkalimeter.

f. Condenser, 400-mm jacket, with one-hole, size 5 stopper at exit end and glass tubing inserted through stopper. It is slanted at about a 30° angle (with exit end at top) and attached to a 24-in.-rod support stand with utility clamp. Tygon tubing is attached to water source and drain, with water entering lower part.

g. Alkalimeter (optional) replaces all items above except d and e.

h. Gas-washing bottle, containing 100 ml concentrated sulfuric acid.

i. Stopcock, hard rubber (optional, helps prevent backflow of solutions).

j. Gas-washing bottle containing 100 ml silver sulfate solution.

k. Filtering flask, 50-ml, with one-hole, size 5 stopper and glass tubing inserted through stopper. This item serves as a liquid trap.

l. Drying bulb containing magnesium perchlorate; a small layer of glass wool is beneath and above the magnesium perchlorate.

m.Rotameter with the sapphire float between the 4.6 and 6.8 levels to afford a flow of 200 to 410 cc per min.

n. Two Nesbitt bulbs (for carbon dioxide absorption) assembled in series. These may be put in test tube basket. A 1/2-in. layer of glass wool is placed in the bottom of the bulb. Indicarb is then added to a point 3/4 in. from the shoulder of the bulb. The Indicarb is covered by a 1/4-in. layer of activated alumina. Introduce sufficient glass wool to reach the neck area and cotton in hollow stopper. For highly reactive materials or materials with high carbonate carbon content, more than two Nesbitt bulbs may be necessary.

o. Filtering flask, 250-ml, with one-hole, size 6 stopper and glass tubing inserted through stopper. This item serves as a particulate trap; it is connected to the vacuum source and regulator with vacuum-pressure tubing.

p. Argyle-bubble tubing, used between glass tube, funnel, 250-ml Erlenmeyer flask, 125-ml Erlenmeyer flask, condenser, gas-washing bottles, 50-ml filtering flask, drying bulb, and rotameter.

q. Rubber tubing used between rotameter, Nesbitt bulbs, and 250-ml filtering flask.

r. Kel-F # 90 stopcock grease is used for all ground glass connections.

Conditioning the train.

Unlike most trains, the carbonate-carbon train requires very little conditioning. If the train has remained idle overnight, the analyst conditions the train by allowing room air to flow through the system at 200 to 410 cc per min for about 5 min (see procedure).

REAGENTS

Chemical Requirements

The following chemicals are ACS reagent grade:

1. Activated alumina, indicating, 8-14 mesh (Fisher #A-545)
2. Ascarite (sodium hydroxide in asbestos) 8-20 mesh
3. Glass wool
4. Sulfuric acid, concentrated
5. Silver sulfate
6. Magnesium perchlorate
7. Indicarb, 6-10 mesh (Fisher #1-181) may be substituted by Ascarite
8. Stopcock grease, Kel-F #90 (Sargent #S-77346)
9. Hydrochloric acid, concentrated
10. Calcium carbonate

Preparation of Solutions

1. Silver sulfate solution: Dissolve about 20.0 g Ag_2SO_4 in 100 ml (use graduate) of concentrated H_2SO_4 by putting both in a 250-ml beaker and heating on a hot plate. Use glass rod to break up and mix Ag_2SO_4.

   Note: Ag_2SO_4 decomposes at 652 C. Allow solution to cool to near room temperature before putting it into gas-washing bottle. Final volume should be about 100 ml.

2. Dilute hydrochloric acid solution (1:4). Using a 500-ml graduated cylinder, put 1,600 ml of distilled water into a 2,000-ml bottle. Add 400 ml of concentrated HCl and mix solutions by shaking and inverting the bottle.

   Note: Prepare new solutions whenever used solutions foam excessively or when analyses of standard plus impurities indicate depletion of silver sulfate effectiveness.

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SAFETY PRECAUTIONS

Follow general laboratory safety rules. This method has no pronounced safety hazards. Care must be taken, however, when handling the concentrated acids.

SAMPLE PREPARATION

The details of sample preparation procedures that describe the drying and grinding techniques are not discussed in this laboratory procedural report. In general, raw refuse, incinerator residue (organics or combustibles), and compost are dried at 70 C to a constant weight and ground to a particle size of less than 1 to 2 mm. Incinerator residue (fines or noncombustibles) and fly ash samples are dried at 105 C to a constant weight and pulverized to pass through a #60 (0.25-cm) sieve. Most of the metals, glass, and ceramics are removed before the samples are ground or pulverized. All samples must be well mixed before aliquots are removed for analysis.

PROCEDURE

Blanks

Increase in weight of the absorption bulbs is due to 1) sample-acid reaction, 2) sample flask and/or acid contamination, 3) atmospheric contamination during sample container's connection to train, and 4) incomplete removal of carbon dioxide from the room air. The blank analyses determine the effects of all the above factors, except sample-acid reaction, on the weights of absorption bulbs. These blank analyses are conducted like the regular sample analyses, except that no sample is used.

The analyst is advised to perform triplicate tests and repeat the blank analyses with each new batch of dilute hydrochloric acid solution.

Standard

The analyst should periodically check the carbonate-carbon train by analyzing a standard (commonly calcium carbonate, previously dried at 105 C for 1 hr). The procedure for analyzing a standard is the same as described for sample analyses. Analyze, however, only 0.2 to 1.0 g of calcium carbonate since this standard reacts quickly and easily with acid. A larger than 1.0 sample of calcium carbonate reacts too violently and causes gas to escape through the separatory funnel and into the room.

Samples

The following procedure applies to all solid waste materials as well as to blanks and standards with the previously mentioned changes.

The analyst is advised to use 1.0- to 5.0-g portions of samples for analysis. Sample weights up to 10 g may be used if nonuniformity of sample warrants. Since the density of raw refuse, compost, and incinerator residue (organics or combustibles) samples is low, the analyst need weigh only 1.0- to 3.0-g portions of such samples.

Procedure	Comments
1. Transfer at least 1 to 5 g (or 1 to 3 g) of a solid waste sample into each of two 250-ml Erlenmeyer flasks. Determine and record the weight of each sample portion to the nearest 0.0001 g.	1. a) Duplicate determinations are sufficient for all solid waste samples. (See Method Evaluation.) b) The type of Erlenmeyer flask depends on whether or not the Knoor alkalimeter is employed.
2. With water going through the condenser, and the stopcock of the separatory funnel open, allow room air to flow through the carbonate-carbon train at 200 to 410 cc per min. (If stopcock is employed between gas-washing bottles, it must be open.)	2. a) The sapphire float in the rotameter should be between the 4.6 and 6.8 levels. b) If conditioning of the train is needed, the air flow is maintained for about 5 min. c) An empty, 250-ml Erlenmeyer flask is used whenever a sample is not being analyzed.
3. Remove the absorption bulbs from the train and close each bulb to the atmosphere. Note: To prevent room air from entering the bulbs, disconnect bulb nearest vacuum source first.	3. A test tube basket is handy for carrying absorption bulbs. Bulbs may be left in basket while connected to the train.
4. Determine and record the weight of each absorption bulb. These weights represent the initial weights of the absorption bulbs.	4. a) The bulbs should be near room temperature before being weighed. The reaction of CO_2 with the Indicarb produces heat. b) Before weighing, each bulb is momentarily vented to the atmosphere and wiped clean with a lint-free cloth or glove. c) Use an analytical balance with a 200-g capacity and 0.1-mg readability.
5. After opening the absorption bulbs to permit gas flow, quickly return the bulbs to the train assembly.	5. Start with the bulb furthest from the vacuum source. The connection of the bulbs to the vacuum source should be performed last.
6. After the rotameter indicates that 200 to 410 cc per min of gas is flowing through the train, remove the empty 250-ml Erlenmeyer flask and connect a similar flask containing the weighed sample to be analyzed.	6. The sapphire float in the rotameter should be between the 4.6 and 6.8 levels.
7. Close the stopcock of the separatory funnel.	7. This stopcock cannot be closed for several minutes.

8. With separatory funnel stopper removed, add 50 ml (using 50-ml graduated cylinder) of dilute HCl solution (1:4).	8. If a Knorr apparatus is used, the drying tube atop the funnel must be removed to add the acid solution.
9. Replace separatory funnel stopper.	
10. By turning stopcock, slowly add dilute acid to 250-ml Erlenmeyer flask.	10. If the sample is calcium carbonate or very high in carbonate, addition of acid to the sample is very, very slow.
11. As soon as all the acid has entered the flask, close stopcock of funnel.	11. If stopcock is not closed immediately, there may be a loss of CO_2.
12. Apply heat until sample and acid solution starts to boil.	12. Be sure all the acid solution comes into contact with the sample. (Flask may need swirling.)
13. With heat removed, slowly open stopcock of funnel, keeping rotameter float between the 4.6 and 6.8 levels.	13. The flow rate should be maintained between 200 and 410 cc per min.
14. With the stopcock completely open and the flow rate adjusted, set a timer for 20 min.	14. A 20-min flush time is sufficient for every type of sample.
15. After the 20-min flush time, stop the flow by removing the rubber tubing from the last absorption bulb. Note: If a stopcock is used between gas-washing bottles, it must be closed.	15. If another connection is separated first, room air will enter the absorption bulbs.
16. Remove all the absorption bulbs from the train and close each to the atmosphere.	16. If necessary, the train may remain idle for several hours.
17. As before, determine and record the weight of each absorption bulb.	17. This weight represents the final weight of each absorption bulb and is used as the initial weight of each bulb for the next sample.
18. If another sample is to be analyzed, repeat the procedure starting with step 5. (If stopcock is employed between gas-washing bottles, it must be open.)	
19. If the train is not to be used for the remainder of the day, turn off water supply to condenser, close vacuum source, shut off gas, and close all stopcocks.	

CALCULATIONS

Standards

Employ the following formula to calculate the theoretical concentration of carbon in a standard sample:

$$\% C = \frac{(N)(F)(100)}{(S)\ (P)}$$

where

% = The percent by weight
C = The element carbon
N = The number of atoms of the element in a molecule of the standard
F = A factor derived by dividing the gram-atomic weight of the element by the gram-molecular weight of the standard
S = The weight of the total sample
P = The decimal fraction representing the concentration of the standard compound in the total analyzed sample (Note: this decimal fraction is the only fraction containing the component for which the sample is being analyzed.)

Example:

Pure Calcium Carbonate $CaCO_3$

$$\% C = \frac{(1)\left(\frac{12.01}{100.09}\right)(100)}{(1.0000)\ (1.00)} = 12.01$$

When the impurities listed on the bottle are considered, the calculated percent of carbon in ACS grade calcium carbonate is still 12.01. (ACS grade is about 99.956 percent pure.)

Samples

Employ the following formula to calculate the concentration of carbon in a solid waste sample:

$$\% C = \frac{(A - B)(X)(100)}{(S)}$$

where

% = The percent by weight
C = The element carbon
A = The sum total increase in the weight of the absorbing bulbs as determined in the unknown analyses
B = The sum total increase in the weight of the absorbing bulbs as determined in the blank analyses
X = A factor derived by dividing the gram-atomic weight of carbon by the gram-molecular weight of carbon dioxide; i.e., (12.01) ÷ (44.01) = 0.2729
S = The weight of the total sample

METHOD EVALUATION

The accuracy of this method was established by analyzing ACS grade calcium carbonate eight times. The average percent of carbon found was 11.97. (The calculated percent of carbon value is 12.01.) The standard deviation for these eight observations was 0.18. This method can analyze solid waste materials containing various forms of carbon or excessive amounts of interferences to within 0.02 to 0.50 of the actual percent of the established value, depending on the type of sample.* For the forms of carbon, sucrose and urea were selected to represent organic carbon. Graphite was employed as elemental form of carbon. Potassium fluoride, sodium sulfate, sodium chloride, and sodium nitrite were employed as the interfering materials. This method should not be employed to analyze samples that contain less than 0.01 percent carbonate carbon.

The precision (pooled standard deviation) of this method was determined by analyzing in triplicate a number of solid waste samples of various types. The pooled standard deviation of the observations for each type of solid waste was calculated using an Olivetti Underwood Programma 101. The calculations revealed that in the analyses of each type of waste, the duplicate and triplicate determinations were about equally precise (Table 1). To ensure precision, the particle size of the samples must be less than 2 mm (or pass through a #60 sieve) and thoroughly mixed before analyzing.

TABLE 1
STANDARD DEVIATION* OF THE CARBON (CARBONATE) DETERMINATION ON CALCIUM CARBONATE AND SOLID WASTES

Type of sample	Number of samples	Carbon		Percent carbon (range)
		Duplicates	Triplicates	
Calcium carbonate†	3	---	0.18	---
Residue:				
Fines‡	12	0.01	0.02	0.05 to 0.69
Organics§	4	0.18	0.15	0.59 to 8.00
Fly ash	6	0.13	0.15	0.70 to 1.39
Raw refuse	13	0.08	0.07	0.06 to 0.96

*A variance estimate can be calculated from the duplicate (or triplicate) set of observations for each sample. The pooled variance is essentially an average of all such estimates for samples of a given type. It is assumed that a single, underlying variance exists for all samples of a given type. The pooled variance is then the best estimate of this underlying variance. The pooled standard deviation is the square root of the pooled variance and is used to estimate the underlying standard deviation.

†ACS grade.

‡Fines are materials remaining after most of the readily combustible substances have been removed by manual sorting. The sorting was performed at the incinerator sites and a 1/2-in. sieve was employed to assist in the separation.

§Organics or combustibles are mostly the readily combustible materials. Unlike fines, these materials are usually retained on a 1/2-in. sieve.

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*Types of solid wastes in this paper refer only to solid samples (domestic origin) such as raw refuse, incinerator fly ash, incinerator residue, and compost.

With this method, the analyst normally uses a 1- to 5-g sample, but he is not restricted to this amount. Because of the difficulties in preparing a very uniform sample, sample weights below 1 g have been found inadequate when analyzing solid waste materials. But samples up to 10 g have been analyzed with no difficulty. The extra sample weights, however, add little to the precision of this method.

The performance of this method requires only periodic attention by the analyst. At best, eight samples a day can be analyzed in duplicate (about 30 min per determination), leaving the analyst with approximately 2 hr of free time per day.

ACKNOWLEDGMENTS

The author wishes to thank the Division of Technical Operations, Office of Solid Waste Management Programs, for providing samples from incinerators. The author also gratefully acknowledges the contribution of Israel Cohen, Solid Waste Research Laboratory, who prepared many of the samples used in developing this method.

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EXTENSION OF CARBON-HYDROGEN METHOD TO INCLUDE DETERMINATION OF VOLATILES OR LOSS ON IGNITION (L.O.I.) AT 950 C*

Donald L. Wilson†

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*This method is intended to be used in conjunction with "Laboratory Procedure for the Gravimetric Determination of Carbon and Hydrogen in Solid Wastes" included in this Manual.

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DISCUSSION

The oxygen content of solid waste samples is found indirectly from the volatile analysis (1). The commonly used method (2, 3) of determining volatiles for solid waste samples presents an awkward means of finding the oxygen content, however. The standard volatile-ash method for solid wastes samples involves heating the sample to 600 C; but, at this temperature, the oxygen combined in carbonates is partially lost because of decomposition of carbonates. Additional analyses (4) that determine carbonate content of the samples and ash after volatilizing must therefore be performed to determine the degree of decomposition of carbonates during volatilization.

A much easier approach to finding the oxygen content of solid wastes samples is to volatilize the samples at a temperature at which almost all of the carbonates decompose. Although the temperature in the regular volatile-ash method can be raised, a carbon-hydrogen method (5) for solid waste samples provides a quick and easy means of determining volatiles at 950 C, a temperature at which most carbonates, especially the most commonly present calcium carbonate, are decomposed.

The carbon-hydrogen method requires that a combustion aid (iron chips) be added to the sample. This combustion aid oxidizes and increases in weight during the carbon-hydrogen analyses. The percent of weight increase that is due to oxidation must be determined for each new batch (usually 5 lb) of iron chips. The increase in weight of the iron chips is then subtracted from the weight of the ash remaining after the carbon-hydrogen analyses to obtain the true weight of the material volatilized.

The terms volatile and ash are commonly used in solid waste management, but they are often misunderstood and misleading. A more meaningful term, which will be used in this method, is loss-on-ignition (L.O.I.) at a particular temperature (950 C in this procedure).

APPARATUS

The apparatus for this method is the same as that described in Reference 5. Note that in the carbon-hydrogen procedure, samples are retained in clay or nickel boats; but since nickel boats may undergo weight changes during the carbon-hydrogen tests, only clay boats may be used in this procedure.

REAGENTS

The chemical requirements and the preparation of reagents for this method are the same as those described in Reference 5.

SAFETY PRECAUTIONS

The safety precautions are the same as those outlined in Reference 5. No additional hazards are involved in this method.

SAMPLE PREPARATION

The techniques of sample preparation for this extension of the carbon-hydrogen method are the same as those mentioned in Reference 5.

PROCEDURE

This procedure applies to all solid waste materials that are analyzed for their carbon-hydrogen contents. Since the mechanics for this procedure are nearly the same as those outlined in the procedure for carbon-hydrogen contents, only the details pertinent to the analysis L. O. I. at 950 C are discussed here.

Procedure	Comments
1. Transfer at least 0.2 to 0.5 gram of DRIED iron chips into each of 10 previously ignited, dry, clay combustion boats. Determine and record the total weight of each sample plus boat to the nearest 0.0001 g.	1. a) This weight represents the normal amount of combustion aid used in the carbon-hydrogen method. b) Minimize handling of boats to prevent contamination. c) Lids to boats are not needed. d) Keeping the boats in a particular order will prevent mix up of samples. e) Boats should be kept in a desiccator until used. f) As needed, dry a small number of iron chips at 105 C and store in a screw-top bottle.
2. Store each boat containing a sample in a desiccator until it is transferred to the combustion tube.	2. It is convenient to use a stiff asbestos pad to support the boats while in the desiccator and during transfer from one place to another.
3. Analyze for blanks by the carbon-hydrogen method, but put each boat with ash in a desiccator after the analysis. Note: Ash from samples high in carbonates may easily absorb moisture from the atmosphere.	3. a) Develop a technique of handling a boat after the carbon-hydrogen analysis. b) Care must be exercised not to spill the ash content of each boat. c) The carbon-hydrogen data obtained here may be used as blank values for the carbon-hydrogen analyses.
4. After each boat has cooled to room temperature, determine and record the weight of each boat plus contents to nearest 0.0001 g.	4. Data are now available for calculating the percent of weight increase of the iron chips (see Calculations).
5. Analyze solid waste samples for their carbon-hydrogen contents by the regular method, but for each sample (a) determine and record the weight of iron chips used to the nearest 0.0001 g, and (b) determine and record the weight of each boat plus contents to the nearest 0.0001 g. after each analysis. Note: Use only clay boats.	5. a) Since the weight of the sample plus clay boat is known, weight of the iron chips may be determined by reweighing the boat plus contents after iron chips are added to and mixed with the sample. b) Data are now available for calculating L. O. I. at 950 C for each sample analyzed (see Calculations).

STANDARDIZATION

Standards are analyzed in the manner described under "Procedure" in Reference 5. Standards for L. O. I. at 950 C may be established by repeated analysis of various solid waste samples or by preparing a sample containing a definite amount of an inert material (pulverized ceramics, for example).

CALCULATIONS

Formula for percent weight increase of iron chips:

$$W_i = \frac{(A - B)(100)}{B - X}$$

where

W_i = percent of weight increase of iron chips
A = total weight of each boat plus iron chips after combustion in the carbon-hydrogen method
B = total weight of each boat plus iron chips before combustion in the carbon-hydrogen method
X = weight of each boat
$(B - X)$ = weight of iron chips before combustion

Note: The percent of weight increase for one case of 10 samples averaged 21.8 percent and ranged from 17.7 to 24.6 percent.

Formula for L. O. I. at 950 C:

$$\text{\% L. O. I. at 950 C} = \frac{[(D - A) + (C)(W_i)]\,(100)}{D - B}$$

where

D = total weight of each boat, sample, and iron chips before combustion in the carbon-hydrogen method
A = total weight of each boat plus contents after combustion in the carbon-hydrogen method
C = weight of iron chips (before combustion) used with each sample

Note: All data are on a dry basis.

METHOD EVALUATION

The data from this method are comparable with data obtained by muffling a solid waste sample at 950 C for 1 hr in air (3). Replicates generally show better agreement with this method than with the muffling method, probably because of the greater temperature control in this method.

Once the percent of weight increase of the iron chips has been established, this method adds about 5 to 10 min extra time to each carbon-hydrogen test.

Metals not removed during sample preparation (7) could interfere with the accuracy of this analysis. The L. O. I. at 950 C could be even less than that at 600 C since more metals could oxidize at the higher temperature and with the pure oxygen atmosphere.

ACKNOWLEDGMENT

The author wishes to thank James U. Doerger for performing the laboratory tests necessary to establish this method.

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MATHEMATICAL DETERMINATION OF TOTAL OXYGEN IN SOLID WASTES

Donald L. Wilson*

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DISCUSSION

The oxygen analysis of solid waste in one of the important ultimate analyses necessary to determine the efficiency of operation of an incinerator, the design of furnaces for incineration, and a complete materials balance of incoming and outgoing material. The oxygen content of solid waste samples must be known if their calorific values are to be calculated from ultimate analyses.

The direct approach of determining oxygen content in solid wastes would involve much time and expense. Although a direct method is more exacting, an indirect approach (1) that employs a formula with ultimate analyses and an ash value is presently being used for each coal or coke sample and can be applied to solid waste samples. The formula must be modified, however, before it can be applied to solid waste samples, since the oxygen formula applies to coal and coke samples and is related to a particular method of ashing (2).

The modified method (3) is actually two oxygen formulas, each of which depends on the ashing technique employed. One procedure of ashing is the standard method for solid waste samples (4). It involves heating the sample to 600 C, but requires testing for the decomposition of carbonates. The other ashing technique is to weigh the ash from the carbon-hydrogen method (5). Although either ashing technique may be used for this method, ashing as part of the carbon-hydrogen method is recommended because the extension of the carbon-hydrogen procedure (weighing the residue and correcting for oxidation of combustion aid) requires very little extra time or effort and eliminates the need for determining the amount of carbonate decomposition.

Total oxygen content of a solid waste sample is defined either as all the oxygen contained in the volatile-at-600-C portion plus the inorganically combined oxygen of carbonates in the sample, or as all the oxygen contained in the volatile-at-950-C portion. This total oxygen value does not include oxygen that is already combined with metals or silicon.

The recommended procedure for determining carbonate oxygen and organically bonded oxygen is first to determine carbonate carbon, to multiply this answer by 4 for carbonate oxygen, and then to obtain the organically bonded oxygen content by subtracting the carbonate oxygen value from the total oxygen concentration.

The two new formulas for calculating total oxygen content in solid waste samples correlate data from as many as eight different analyses. The eight components involved are: total carbon (5), carbonate carbon in total sample (6), carbonate carbon in ash from volatile at 600 C, total hydrogen, total nitrogen (7), total sulfur (8), total chlorine (10), and a value for volatile or ash at 600 C or 950 C. The oxygen method described here deals with the procedures for the eight related components.

The new formulas for oxygen concentrations still have the errors inherent in the other analyses, particularly the volatile analysis; however, they do take into account carbonate oxygen, which could cause the greatest inaccuracy in total oxygen data for solid waste samples.

APPARATUS

This procedure requires no apparatus other than that needed to perform the eight different analyses that provide the data for this method.

REAGENTS

No reagents are necessary for this analysis. Reagents are needed, however, for the eight correlated methods and are mentioned under the appropriate method.

SAFETY PRECAUTIONS

No safety hazards exist in this procedure. Safety precautions necessary in related methods are discussed under those methods.

SAMPLE PREPARATION

Preparation of solid waste samples are discussed in Reference 11 and elsewhere.

PROCEDURE

Carbon-Hydrogen Analyses

Procedure	Comments
1. Determine total carbon and hydrogen contents by the method developed particularly for solid wastes samples, Reference (5).	1. Larger sample portions are analyzed with this method than with other similar ones.

Carbonate Carbon Analysis

Procedure	Comments
1. Determine carbonate carbon content by the method developed particularly for solid waste samples, Reference (6).	1. a) This analysis is needed for the carbonate oxygen content of the sample. b) This same method is employed to find carbonate carbon in ash from the volatile-at-600 C analysis.

Nitrogen Analysis

Procedure	Comments
1. Nitrogen content of solid waste samples may be determined by an AOAC method (7).	1. This method is the most commonly used for solid waste samples.
2. Nitrogen content of incinerator raw refuse, combustible incinerator residue, and compost samples may be estimated to be 0.75 percent (3).	2. This estimate is the average nitrogen content of 12 raw refuse samples. The nitrogen contents ranged from 0.49 to 1.42 percent.
3. Nitrogen content of noncombustible incinerator-residue samples and incinerator fly ash samples may be estimated to be 0.15 percent (3).	3. This estimate is the average nitrogen content of nine noncombustible-residue samples. The nitrogen concentrations ranged from 0.04 to 0.31 percent.

Sulfur Analysis

Procedure	Comments
1. Sulfur content of solid waste samples may be determined by an ASTM method (see References 8 and 9).	1. a) This is the most commonly used method for solid waste samples (see Reference 8). b) The related bomb-combustion method was developed especially for solid waste samples (see Reference 9).
2. Sulfur content of solid waste samples (raw refuse, incinerator residue, incinerator fly ash, and compost) may be estimated to be 0.20 percent (3).	2. The exact average sulfur content of 21 samples (12 raw refuse and nine noncombustible-residue samples) was 0.19 percent. The sulfur concentrations ranged from 0.10 to 0.37 percent.

Chlorine Analysis

Procedure	Comments
1. Chlorine content of solid waste samples may be determined by an AOAC method (10).	1. This method is the most commonly used for solid waste samples.
2. Chlorine content of incinerator raw refuse, combustible incinerator residue, and compost samples may be estimated to be 0.50 percent (3).	2. The exact average chlorine content of 12 raw refuse samples was 0.52 percent. The chlorine concentrations ranged from 0.29 to 1.10 percent.
3. Chlorine content of noncombustible incinerator residue samples and incinerator fly ash samples may be estimated to be 0.10 percent (3).	3. The exact average chlorine content of nine noncombustible residue samples was 0.11 percent. The chlorine concentrations ranged from 0.06 to 0.15 percent.

Volatile-Ash Analysis at 600 C

Procedure	Comments
1. The volatile or ash content of solid waste samples at 600 C may be determined by an APWA method (3, 4).	1. a) This method is the most commonly used for solid waste samples, but it is not preferred. b) Although it is not commonly used in solid waste management, the term L.O.I. (loss-on-ignition) at 600 C is more meaningful than the term volatiles. c) Data obtained with this method do not vary with the age of samples, which are up to 2 years old. d) Reproducibility of data is affected by large amounts of carbonates present in the sample.

2. Determine carbonate carbon content of the ash from the volatile analysis (see Carbonate Carbon Analysis).	2. Carbonate carbon analysis is needed in order to determine the degree of decomposition of carbonate in the analysis of volatile content at 600 C.

Volatile-Ash Analysis at 950 C

Procedure	Comments
1. The volatile or ash content of solid waste samples at 950 C may be determined either by muffling, as in the APWA method for 600 C, or by weighing the ash from the carbon-hydrogen method (3, 4, 12).	1. a) This analysis (volatiles at 950 C) is not the most commonly used for solid waste samples; but it is preferred because it is easily obtained with the carbon-hydrogen method, and the decomposition of carbonates may be considered to be 100 percent at 950 C. b) Although it is not commonly used in solid waste management, the term L.O.I. (loss-on-ignition) at 950 C is more meaningful than the term volatiles.
2. If the ash data are obtained from the carbon-hydrogen method, the ash value must be corrected for the increase in weight of the combustion aid used (iron chips). The average percent increase in weight of the combustion aid is 21.8 percent.	2. a) Since the ash may contain calcium oxide, which easily absorbs moisture, the ash from the carbon-hydrogen must be kept in a desiccator until weighed. b) This average percent of weight increase was determined from 10 analyses with iron chips. The weight of iron chips varied from 0.1957 g to 0.4192 g. The percent of weight increase varied from 17.7 to 24.6 percent.

STANDARDIZATION AND CALIBRATION

No standardization and calibration are required for the total oxygen analysis. These techniques are described in the methods associated with this procedure for determining total oxygen content of solid waste samples.

CALCULATIONS

1. Formula for total percent oxygen from volatiles at 600 C:

$$\% O = V_1 - \left(\frac{44}{12}\right)(C_i)(C_{id}) - C_o - H - N - S - Cl + \left(\frac{4}{1}\right)(C_i)$$

where

% = the total percent by weight
O = the element oxygen
V_1 = the percent of sample that volatilized at 600 C

$\frac{44}{12}$ = the molar ratio of carbon dioxide to carbon
C_i = the percent of inorganically bonded (carbonate) elemental carbon
C_{id} = the decimal fraction of the amount of carbonate carbon that decomposed in the volatile-at-600-C analysis
C_o = the percent of organically bonded elemental carbon
H = the total percent of the element hydrogen
N = the total percent of the element nitrogen
S = the total percent of the element sulfur
Cl = the total percent of the element chlorine
$\frac{4}{1}$ = the molar ratio of oxygen to carbon in carbonates

Note: N, S, and Cl may be estimated if not actually determined. All results are on a dry basis.

2. Formula for total percent oxygen from volatiles at 950 C:

$$\% O = V_2 - C_t - H - N - S - Cl$$

where

% = the total percent by weight
O = the element oxygen
V_2 = the percent of sample that volatilized at 950 C
C_t = the total percent of the element carbon

Note: N, S, and Cl may be estimated if not actually determined. All results are on a dry basis.

3. Conversion of carbonate carbon analysis of ash from volatile at 600 C to original sample basis:

$$\% C_{ie} = (C_{ia})(A)$$

where

% = the percent by weight
C_{ie} = the carbonate carbon in ash at 600 C, on original sample basis (before ashing)
C_{ia} = the percent of carbonate carbon in ash at 600 C
A = the decimal fraction of the amount of ash remaining after volatilizing the sample at 600 C

Note: All results are on a dry basis.

4. Formula for the amount of decomposition of carbonates during the volatile-at-600-C analysis:

$$\% C_{id} = \frac{(C_i - C_{ie})(100)}{C_i}$$

Note: All results are on a dry basis.

5. Conversion of data from a wet basis to a dry basis:

$$\% X_d = (100)(\% X_w) \div (100 - m)$$

where

$\%$ = the percent by weight
X_d = the ingredient on a dry basis, except volatiles
X_w = the ingredient on a wet basis, except volatiles
m = the percent of moisture (loss at 105 C)

Volatiles only:

$$\%V_d = (V_w - m) \div (100 - m)$$

where

$\%$ = the percent by weight
V_d = the volatile portion of the sample, dry basis
V_w = the percent volatile portion of the sample, wet basis

6. Formula for percent carbonate oxygen:

$$\%\ O_c = (\tfrac{4}{1})\ (C_i)$$

where

$\%$ = the percent by weight
O_c = inorganically bonded (carbonate) elemental oxygen

7. Formula for percent organically bonded oxygen:

$$\%\ O_o = \%\ O - \%\ O_c$$

where

$\%$ = the percent by weight
O_o = organically bonded elemental oxygen

METHOD EVALUATION

The accuracy of this method is dependent on the accuracy of the related analyses and the care taken in removing metals during sample preparation.

The variance in the oxygen results is a composite of the precision of the associated analyses. The largest deviation from the average value exists usually in the volatile analysis; replication of the volatile data is therefore the major factor in determining the precision of the oxygen data. Since the carbonate content of a sample affects the replication of the volatile-at-600-C analysis, the oxygen data of samples high in carbonate content can be expected to show poor precision if the data were obtained with the volatile-at-600-C analysis.

An inspection of past data indicates that the oxygen value could, at most, vary 1 or 2 actual percent from the average. This is well within limits of requirements for an estimated oxygen value of solid waste samples.

The time required to perform the calculations for the oxygen value is trivial, but the related laboratory analyses required are very time consuming. Such analyses are generally not performed just for an oxygen value, however. The breakdown of total carbon data into organic and carbonate carbon and the decomposition of carbonates in the volatile-at-600-C analysis are necessary information for calculating oxygen values, but the concepts have also been useful in producing a better understanding of the nature of solid waste samples and of the analytical tests performed on them.

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MATHEMATICAL DETERMINATION OF TOTAL HEAT OF COMBUSTION CONTENT OF SOLID WASTES

Donald L. Wilson*

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DISCUSSION

The heat contents of various solid wastes materials (usually expressed as: British thermal units (Btu) per pound of sample) are important for establishing an energy balance about an incinerator and for determining its efficiency. The heat values of solid waste, and particularly of raw refuse, are also considered when planning the design of an incinerator. The heat contents of incinerator residue and compost used for landfill are essential data for determining the stability of these waste products.

The experimental method (1, 2) of determining heat contents of prepared solid waste samples (3) is sometimes difficult to perform, and the accuracy of the data is at times questionable. The heat contents of such samples may be determined mathematically by a modified Dulong formula (4) with data from ultimate analysis. This mathematical approach can either eliminate the need for an experimental test or verify the accuracy of such a test.

The formula for calculating the Btu-per-pound content of solid waste samples correlates data from 14 different analyses. The six directly employed components are (a) organic carbon (5, 6), (b) total hydrogen (5), (c) organic oxygen (6, 7), (d) total nitrogen (8), (e) total sulfur (2, 9), and (f) carbonate carbon (6). The eight related analyses are (a) total carbon (5), (b) carbonate carbon in ash for volatile at 600 C (6, 7), (c) decomposition of carbonates at 600 C (6, 7), (d) volatile at 600 C value (7, 10), (e) volatile at 950 C value (7, 11), (f) total oxygen (7), (g) carbonate oxygen (6, 7), and (h) total chlorine (7, 12). The heat of combustion method described here deals with the procedures for the six directly related components.

The modified Dulong formula still has the errors inherent in the other analysis; however, the agreement between experimental and mathematical Btu-per-pound values is about equal to the agreement between replicate experimental Btu-per-pound values (4).

Total heat of combustion content of a solid waste sample is defined as the oxidation of organic carbon to carbon dioxide, hydrogen to water, nitrogen to nitrogen dioxide, sulfur to sulfur dioxide, and the decomposition of carbonates present in the sample.

APPARATUS

This procedure requires no apparatus other than what is needed for the 14 different analyses that must be performed to obtain the data for this method.

REAGENTS

No reagents are necessary for this analysis. The reagents are needed for the 14 correlated methods and are mentioned in the appropriate method.

SAFETY PRECAUTIONS

No safety hazards exist in this procedure. Safety precautions necessary for related methods have been discussed in those methods.

SAMPLE PREPARATION

Preparation of solid wastes samples are discussed in the carbon-hydrogen method (5) and elsewhere (3). Therefore, sample preparation techniques are not repeated in this method.

PROCEDURE

Carbon-Hydrogen Analyses

Procedure	Comments
1. Determine total carbon and hydrogen contents by the method developed particularly for solid wastes samples (5).	1. a) Larger sample portions are analyzed with this method than with other similar methods.
Note: L.O.I. at 950 C may be determined at the same time (7, 11).	b) The total carbon value will be used in conjunction with the carbonate carbon value to determine the organically bonded carbon content.

Oxygen Analysis

Procedure	Comments
1. Determine total oxygen content by the method developed particularly for solid waste samples (7).	1. a) This method overcomes difficulties when applying an ASTM oxygen method to solid waste samples.
Note: Chlorine content is determined during this analysis (7, 12).	b) The organically bonded oxygen content of the sample is determined from the total oxygen content and the carbonate oxygen content. The carbonate oxygen content is calculated from the carbonate carbon content.

Nitrogen Analysis

Procedure	Comments
1. Nitrogen content of solid samples is determined by one of the recommended methods for solid waste (8).	1. The Kjeldahl - Wilfarth - Gunning - Winkler Method is the most commonly used method. This method does not, however, include nitrate nitrogen in the total nitrogen data.
2. Nitrogen content of incinerator raw refuse, combustible incinerator residue, and compost samples may be estimated to be 0.75 percent.	2. This estimate is the average nitrogen content of 12 raw refuse samples. These nitrogen contents ranged from 0.49 to 1.42 percent.
3. Nitrogen content of noncombustible incinerator residue samples and incinerator fly ash samples may be estimated to be 0.15 percent.	3. This estimate is the average nitrogen content of nine noncombustible residue samples. The nitrogen concentrations ranged from 0.04 to 0.31 percent.

Sulfur Analysis

Procedure	Comments
1. Sulfur content of solid waste samples may be determined by an ASTM method (2, 9).	1. a) The ASTM method is the most commonly used method (9).

	b) The related bomb-combustion method was developed especially for solid waste samples (2).
2. Sulfur content of solid waste samples (raw refuse, incinerator residue, incinerator fly ash, and compost) may be estimated to be 0.20 percent.	2. The exact average sulfur content of 21 samples (12 raw refuse and nine noncombustible residue samples) was 0.19 percent. The sulfur concentration ranged from 0.10 to 0.37 percent.

Carbonate Carbon Analysis

Procedure	Comments
1. Determine carbonate carbon content by the method developed particularly for solid wastes samples (6).	1. This analysis is also needed to distinguish organic carbon from total carbon, and organic oxygen from total oxygen.

STANDARDIZATION AND CALIBRATION

No standardization and calibration are required for this procedure. These techniques are disclosed in the methods associated with this analysis.

CALCULATIONS

1. Formula for total heat or combustion content (Btu per pound) of solid wastes:

$$\text{Btu per pound} = 14{,}096\ C_o + 60{,}214\left(H - \frac{O}{8}\right) + 1040\ N + 3982\ S$$

$$+ 8929\left(\frac{H - \frac{O}{8}}{2}\right) + 4274\left(\frac{O}{2}\right) - 6382\ C_i$$

where

Btu per pound = British thermal units per pound

14,096 = the heat of combustion (Btu per pound) of graphite carbon

C_o = the decimal percent of organically bonded elemental carbon

60,214 = the heat of formation (Btu per pound) of liquid water (constant volume) from hydrogen and oxygen gases

H = the total decimal percent of the element hydrogen

O = the decimal percent of organically bonded oxygen

$(H - \frac{O}{8})$ = the decimal percent of available hydrogen

N = the total decimal percent of the element nitrogen

S = the total decimal percent of the element sulfur

C_i = the decimal percent of inorganically bonded (carbonate) elemental carbon

Note: N and S may be estimated if not actually determined. All results are on a dry basis (prepared samples).

2. Formula for total percent of organically bonded carbon:

$$\% C_o = \% C - \% C_i$$

where

% = the decimal percent by weight
C = the element carbon (total)

3. Formula for percent of carbonate oxygen:

$$\% O_c = (\tfrac{4}{1})(C_i)$$

where

% = the percent by weight
O_c = inorganically bonded (carbonate) elemental oxygen

4. Formula for percent of organically bonded oxygen:

$$\% O \text{ or } \% O_o = \% O_t - \% O_c$$

where

% = the percent by weight
O or O_o = organically bonded elemental oxygen
O_t = the element oxygen (total)

5. Conversion of data from a wet basis to a dry basis:

$$\% X_d = (100)(\% X_w) \div (100 - m)$$

where

% = the percent by weight
X_d = the ingredient on a dry basis, except volatiles
X_w = the ingredient on a wet basis, except volatiles
m = the percent of moisture (loss at 105 C)

Volatiles only:

$$\% V_d = (V_w - m) \div (100 - m)$$

where

% = the percent by weight
V_d = the volatile portion of the sample, dry basis
V_w = the percent volatile portion of the sample, wet basis

METHOD EVALUATION

The accuracy of this method is dependent upon the accuracy of the related analyses and the care shown in removing glass, ceramics, and metals during sample preparation.

When this procedure was applied to 61 samples from five incinerators (4), the data revealed that

the variation between calculated Btu-per-pound values and experimental Btu-per-pound values has about the same magnitude as the variation between replicate experimental values. These calculated Btu-per-pound values are well within limits of requirements for an estimated Btu-per-pound value of solid waste samples.

The time required to perform the calculations for the Btu-per-pound value is short, but the related laboratory analyses required are very time consuming; such analyses are generally not performed for just a Btu-per-pound value, however. The same ultimate analysis data are used for establishing a material balance about the same incinerator and also for determining the efficiency of that incinerator to reduce the volume of solid waste material.

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THE ALSTERBERG (AZIDE) MODIFICATION OF THE WINKLER METHOD FOR DETERMINING THE BOD OF INCINERATOR QUENCH WATER AND THE CALIBRATION OF THE WESTON & STACK DISSOLVED OXYGEN ANALYZER MODEL 300-B

Donald L. Wilson*

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DISCUSSION

The Winkler method, developed in 1888, is the routine chemical method for the determination of dissolved oxygen (DO). The basic procedure involves the oxidation of manganous hydroxide (Mn^{++}) by the oxygen dissolved in the water to manganic hydroxide:

$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4 \qquad (1)$$

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2 \qquad (2)$$

Manganous hydroxide is a white flocculant precipitate that changes to light brown when oxidized. Since the reaction with the oxygen must occur on the surface of the floc particles, physical mixing at this point is important.

When manganic hydroxide is acidified, manganic sulfate is formed:

$$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O \qquad (3)$$

In the presence of iodide, the manganic salt acts as an oxiding agent, releasing free iodine:

$$Mn(SO_4)_2 + 2KI \rightarrow MnSO_4 + K_2SO_4 + I_2 \qquad (4)$$

The iodine, which is stoichiometrically equivalent to the DO of the sample, is titrated with thiosulfate:

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \qquad (5)$$

For convenience, the alkali (equation 1) and the iodide (equation 4) are combined into a single, alkaline-iodide reagent.

The original Winkler method has been modified since oxidizing agents give a positive interference, reducing agents a negative interference, and organic compounds a varied interference. The most common interference is that caused by nitrites, commonly present in polluted waters and wastes. The nitrite ion reacts with iodides as follows:

$$2NO_2^- + 2I^- + 4H^+ \rightarrow I_2 + 2NO + 2H_2O \qquad (6)$$

$$2NO + 1/2O_2 + H_2O \rightarrow 2NO_2^- + 2H^+ \qquad (7)$$

The Alsterberg (Azide) Modification uses sodium azide to reduce the nitrites in the following manners:

$$NaN_3 + H^+ \rightarrow HN_3 + Na^+ \qquad (8)$$

$$HN_3 + NO_2 + H^+ \rightarrow N_2 + N_2O + H_2O \qquad (9)$$

The Alsterberg (Azide) Modification of the Winkler Method uses prepared dilution water and is employed to standardize the Weston and Stack DO Analyzer. Before the method can be used to determine the BOD of incinerator quench water, however, the samples must be analyzed for the presence of interfering substances. If the final diluted sample contains more than 4 ppm nitrite, 1 ppm ferrous iron, or 200 ppm ferric iron, or any sulfite or thiosulfate, or large amounts of chloride (which may evolve as chlorine during the analysis), the Alsterberg Modification cannot be employed. If, however, the final diluted sample contains less than 4 ppm nitrite, 1 ppm ferrous iron, or 50 ppm ferric iron, valid results can be obtained using the procedure. In addition, samples that contain residual chlorine or 50 to 200 ppm ferric iron, or that have a pH of less than 6.5 or greater than 8.3 can also be evaluated using certain alterations of the procedure.

The preliminary tests presented in the appendix can be employed to evaluate the applicability of the Alsterberg (Azide) Modification in the determination of the BOD of a quench water sample. Since the tests are qualitative and involve distinct color changes or precipitates, the concentration of the reagents may be approximate. Standards should be employed, however, to verify the validity of the preliminary analyses and to give the analyst some knowledge of the color intensity and hue.

Since the tests are not specific and hence give a positive reaction in the presence of several substances, they are assigned group numbers. Each of the interfering substances mentioned earlier does, however, fall into one of the groups.

APPARATUS

All glass and plastic apparatus must be cleaned thoroughly to ensure the removal of all materials capable of exerting a BOD. Detergents may be used if cleaning is followed by thorough rinsing with distilled water.

The apparatus requirements are as follows:

1. Incubation (BOD) bottles, 300-ml capacity
2. Air incubator or water bath, thermostatically controlled at 20 C ± 1 C
3. Buret, 50-ml
4. Volumetric flasks, 200-ml, specially marked for 203 ml
5. Graduates, 10-ml, 50-ml, 1-liter, and 2-liter
6. Volumetric flasks, 100-ml, 500-ml, 1-liter
7. Mohr pipets, 10-ml with 1-ml divisions
8. Carboys, two 7-liter or more, polyethylene nalgene, wide-mouth
9. Reagent bottles, 1-liter, narrow-mouth, ground glass stoppers
10. Erlenmeyer flasks, 500-ml
11. Beakers, 250-ml, 2-liter, and 3-liter
12. Siphon tubing
13. Balance, analytical (also trip, if available)
14. pH paper, range 2 to 9
15. Sample collection bottles, polyethylene (or similar unbreakable material), narrow mouth, tightly fitting caps, about 1-liter, sterile (no material present that has a BOD value)
16. Magnetic stirrer with Teflon-coated stirring bar
17. Ice chest capable of holding several 1-liter, sample collection bottles and able to maintain a 5 C temperature for 24 hr

REAGENTS

Chemical Requirements

The following chemicals are ACS, reagent grade:

1. Phosphate buffer solution, pH 7.2 (or prepared)
2. Potassium phosphate, monobasic
3. Potassium phosphate, dibasic
4. Sodium phosphate, dibasic, heptahedrate
5. Ammonium chloride
6. Magnesium sulfate, crystal
7. Calcium chloride, anhydrous
8. Ferric chloride, lump

9. Sodium hydroxide or potassium hydroxide
10. Sodium iodide or potassium iodide
11. Sodium azide
12. Sulfuric acid, concentrated
13. Manganese(ous) sulfate, monohydrate (may be other hydrates)
14. Thyodene
15. Sodium thiosulfate, crystalline
16. Chloroform
17. Potassium biniodate, solid or 0.025N solution, or potassium dichromate
18. Potassium fluoride

Preparation of Solutions

All solutions are prepared with distilled water that (a) is distilled from a block-tin or all-glass still, (b) contains less than 0.01 mg per liter copper, and (c) is free of chlorine, chloramines, caustic alkalinity, organic materials, and acids. The solutions are prepared in the following manner:

1. Standard sodium thiosulfate solution, 0.025 N: Dissolve exactly 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and dilute to 1 liter. Preserve by adding 5 ml of chloroform. Note: [a] This solution is equivalent to 0.200 mg DO per 1.00 ml. When employed to titrate 203 ml of treated sample (200 ml of original sample), each ml is then equivalent to 1 mg per liter (ppm) DO in sample. [b] This solution is not stable more than 9 days (see Standardization).
2. Standard potassium biniodate solution, 0.025 N (if not purchased): Dissolve 0.8124g $KHIO_3$ in distilled water and dilute with same to 1 liter.
3. Standard potassium dichromate solution, 0.025 N (if standard potassium biniodate solution is not available): Dissolve 1.226g $K_2Cr_2O_7$ (previously dried for 2 hr at 110 C) in distilled water and dilute with same to 1 liter.
4. Sodium hydroxide solution, approximately 1 N: Dissolve 41.6 g NaOH in distilled water and dilute with same to 1 liter.
5. Sulfuric acid solution, approximately 1 N: Cautiously add 28 ml of concentrated H_2SO_4 to distilled water and dilute with same to 1 liter.
6. Phosphate buffer solution, pH 7.2 (if not purchased): Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl in 500 ml of distilled water and dilute with same to 1 liter. The pH of this buffer should be 7.2 without further adjustment.
7. Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute with same to 1 liter.
8. Calcium chloride solution: Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute with same to 1 liter.
9. Ferric chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute with same to 1 liter.
10. Dilution water: Place 7 liters of distilled water in polyethylene, wide-mouth, carboys (other volumes and containers may be used). Add 7 ml (1 ml per liter of water) of each of the following prepared solutions: Phosphate buffer, pH 7.2; magnesium sulfate; calcium chloride; and ferric chloride. This water should be at 20 C before it is used.

11. Alkali-iodide-azide reagent: Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 liter. To this solution, add 10 g NaN_3 dissolved in 40 ml of distilled water, stirring constantly. This reagent should not give a color with Thyodene indicator when diluted and acidified.
12. Manganese sulfate solution: Dissolve 480 g $MnSO_4 \cdot 4H_2O$ (or 400 g $MnSO_4 \cdot 2H_2O$ or 364 g $MnSO_4 \cdot H_2O$) in distilled water, filter, and dilute to 1 liter.
13. Potassium fluoride solution: Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 ml.

SAFETY PRECAUTIONS

Follow general laboratory safety rules. This method has no pronounced safety hazards. Care must be taken, however, when handling the concentrated sulfuric acid. A pad of tissue held over the BOD bottles when they are inverted will prevent any splattering of the treated sample that might leave stains and harm the analyst's hand.

STANDARDIZATION

With sodium thiosulfate prepared exactly, no standardization is needed. Standard sodium thiosulfate solution, exactly 0.025 N, is equivalent to 0.200 mg DO per 1.00 ml; or when 203 ml of the treated sample is titrated with 0.025 N thiosulfate:

1 ml 0.025 N thiosulfate = 1 mg per liter DO

A sodium thiosulfate solution is not stable, however, and solutions over 9 days old must be standardized with a biniodate (preferred) or dichromate standard 0.025 N solution.

The procedure for standarization is as follows:

Procedure	Comments
1. Dissolve approximately 2 g of KI in a 500-ml Erlenmeyer flask with 150 ml of distilled water.	1. Trip balance may be used if available.
2. Add 10 ml of dilute sulfuric acid.	2. 1 part concentrated H_2SO_4 and 9 parts distilled water.
3. Add 20 ml of standard solution.	3. Biniodate or dichromate.
4. Dilute to 300 ml with distilled water.	4. If dichromate standard solution is used, put flask in the dark for 5 min.
5. Titrate the liberated iodine with standardized thiosulfate titrate to a pale straw color.	
6. Add about 1/2 g of Thyodene indicator and shake flask.	6. After Thyodene is added, a blue color appears.
7. Continue titrating until blue color disappears.	
8. Record the amount of titrant used.	
9. Repeat steps 1 through 8 for a second test.	9. The absolute value of the difference between duplicated readings should not exceed 1.96 $\sqrt{2}s$, or 0.36 ppm, more than 5 percent of the time. See section on Precision.

ANALYSIS OF SAMPLES

Sample Collection

Site selection.

When the BOD of quench water is measured to determine the amount of oxidizable wastes that will be discharged to a sewerage system serving an incinerator facility or to a drainage system associated with the residue disposal area, the site of sample collection must be chosen with due consideration. Settlement tanks, surface pools, sewers and other areas immediately adjacent to the sewerage or drainage system are preferable collection sites.

Sample size and container.

Normally 50 ml of sample is needed to perfrom the BOD analysis; however, since various dilutions may be needed and a larger sample size may be more representative, it is recommended that a 1-liter sample of quench water be collected.

The samples should be collected in sterile, unbreakable bottles with narrow mouths and caps that can be tightly fitted. The sample bottle should be completely filled. All containers must be thoroughly rinsed, especially if cleaned with a detergent, before they can be reused.

Samples should not be collected on Monday or Tuesday unless the analysts are to work on Saturday or Sunday (5-day BOD).

Sample Preservation and Shipment

If the sample analysis is to be initiated within 4 hr after collection, sample preservation measures are not absolutely necessary. If the analysis will be started after 4 hr, however, samples should be placed in an ice chest or similar container soon after collection so that they will be maintained in the dark at 5 C. The bottle caps must be tightly fitted to prevent an increase in oxygen solubility with the reduction in temperature.

Sample shipment to the laboratory should be immediate (via air freight if necessary) to ensure the initiation of BOD analysis in the laboratory within 24 hr of sample collection. Samples received after they are 24 hr old should not be analyzed. Because samples require some preparation before the actual analysis and because the exact dilution requirements may not be known, samples (not at 5 C) should be shipped to the laboratory so that they are received 2 to 4 hr before the end of the normal working day, depending on the number of samples and laboratory personnel. Samples that have been shipped in an ice chest at 5 C and kept under refrigeration may be analyzed the following day provided the analysis can be initiated before the samples are 24 hr old.

Sample and Blank Preparation

Adjustment for nitrification process.

Before analysis, each sample and dilution-water blank is treated as follows to inhibit the nitrification process:

Procedure	Comments
1. Place 50 ml of a thoroughly mixed, quench water sample in a 250-ml beaker.	1. The exact volume of the quench water sample depends on the dilution requirements. (See Dilution and Aeration.)
2. Using pH paper, check the pH of the sample.	2. Usually the pH is about 11.
3. Using 1 N NaOH or 1 N H_2SO_4, adjust the pH of the sample to a range of 2 to 3; maintain pH for 15 min.	3. Omit if the sample already has a pH of 2 to 3.

4. Then neutralize the sample to a pH of 6.5 to 8.3.	4. Employ the same 1 N solutions as in step 3.

Adjustment for residual chlorine.

Since residual chlorine dissipates when samples either stand for 1 to 2 hr or are well aerated, no adjustments are recommended.

Dilution and aeration.

Prepared samples must be diluted in order to obtain a measurable depletion of oxygen (2 ppm to 7 ppm) at the end of the 5-day incubation period. Since incinerator quench water usually has a BOD of 100-300 ppm, a suitable or applicable dilution is 50 ml of sample diluted to 2 liters. If the analyst suspects that the BOD of the quench water differs from the usual value, he should test various dilutions since the analysis cannot be repeated on the same original sample after the 5-day waiting period. To obtain more reliable results, 5 BOD bottles should be prepared: two for the initial DO (can be immediately repeated if necessary) and three for the final DO (only two reasonable results are needed). The final DO values should never be less than 1.0 ppm.

Since the dilution water employed in the analysis of each quench water may contain a few oxidizable materials capable of exerting a small BOD, each quench water analysis should include a blank evaluation, i.e., a determination of the BOD of the dilution water. The observed BOD of the quench water can then be corrected by subtracting the appropriate proportionate fraction of this blank value.

The dilution and aeration procedures are as follows:

Procedure	Comments
1. Pour the total prepared sample from the 250-ml beaker into a 2-liter graduate and dilute to the mark with dilution water.	1. Solution still represents 50 ml of original sample.
2. Aerate the sample by pouring it back and forth from the graduate into a 3-liter beaker at least 3 times.	2. Dilution water blank is aerated in like manner.
3. Siphon the diluted, aerated sample or blank from the beaker into 5 BOD bottles.	3. The sample should be stirred continuously using a magnetic stirrer and a Teflon coated, magnetic bar.
4. The DO concentration of the sample or blank in 2 BOD bottles should be determined immediately.	4. a) See Determination of the DO Concentration. b) At least two reasonable DO results are needed. See section on Precision.
5. Then put the remaining 3 BOD bottles in an incubator (or waterbath) and determine their DO content after a 5-day incubation period at 20 C.	5. a) During the incubation period, the samples should not be exposed to the light. b) See Determination of the DO Concentration. c) Only two reasonable DO results are needed. See section on Precision.

Determination of the DO Concentration

Procedure	Comments
1. To each BOD sample bottle add 2 ml of manganous sulfate solution and then 2 ml of alkaline-iodide-azide reagent.	1. a) When a sample is being evaluated for the purpose of DO probe calibration, the probe determination of the DO content must precede the Winkler determination of that same sample. b) The tip of each pipette must be BELOW the surface of the sample.
2. Replace the stopper, exclude air bubbles, and mix by inverting 9 to 10 times.	2. A Kimwipe pad held over the top of the bottle helps prevent splattering.
3. When the precipitate settles, repeat the inverting and settling.	
4. After there is about 100 ml of clear supernate, remove stopper and immediately add 2 ml of concentrated H_2SO_4 ABOVE the surface of the sample.	4. If the diluted sample contains more than 50 ppm ferric iron, add 1 ml of KF solution before the acid.
5. Stopper the bottle and mix to dissolve the precipitate.	
6. By means of a modified, 200-ml volumetric flask, transfer 203 ml of the treated sample to a 500-ml Erlenmeyer flask.	6. a) Volume adjusted for the 4 ml of reagents added. (See Calculations.) b) Transfer 204 ml if KF solution is used.
7. Titrate the treated aliquot with standardized thiosulfate titrant to a pale straw color.	
8. Add about 1/2 g of Thyodene indicator and shake flask.	8. After Thyodene is added, a blue color appears.
9. Continue titrating until blue color disappears.	
10. Record the amount of titrant used.	
11. Repeat steps 1 through 10 with each BOD sample bottle.	11. a) Titration values less than 1.0 ml should be disregarded. b) The absolute value of the difference between duplicate readings should not exceed $1.96\sqrt{2s}$, or 1.35 ppm, more than 5 percent of the time (See Precision.)

CALCULATIONS

Sample Volume to be Titrated

Since a portion of the sample in the BOD bottle is displaced during the analysis because of the addition of reagents, the volume of sample to be titrated must be adjusted to compensate for this loss. The required volume is calculated as follows:

$$V_1 = 200\left(\frac{V_2}{V_2 - V_3}\right)$$

where

V_1 = The volume of sample to be titrated
V_2 = The volume of BOD bottle employed
V_3 = The volume of reagents added (The volume of the sulfuric acid added is not included since the acid only displaces sample that has been deoxygenated.)

DO Content of Sample

The DO content of a sample is calculated as follows:

$$D = FV_4$$

where

D = The DO content of the sample being titrated
F = The correction factor-ratio of normality of standard (thiosulfate) to 0.025 (normality of biniodate or chromate)
V_4 = The volume of sodium thiosulfate used to titrate the sample

BOD of Sample

Dilution water sample.

The following formula should be employed to calculate the BOD of each individual sample of dilution water.

$$BOD_1 = D_1 - D_2$$

where

BOD_1 = The biochemical oxygen demand of dilution water
D_1 = The DO content of initial (before incubation) dilution water
D_2 = The DO content of final (after incubation) dilution water

Quench water sample.

The initial DO concentration minus the final DO concentration equals the BOD of the diluted sample. The BOD of the diluted sample times the dilution factor equals the BOD of the original sample.

The dilution factor is found by dividing the original amount of sample taken into the final dilution. For example, 50 ml of sample diluted into 2 liters gives a factor of 40.

The following formula should be employed to calculate the BOD of each individual sample of quench water.

$$BOD_2 = F[(D_3 - D_4) - P_1(BOD_1)]$$

where

BOD_2 = The BOD of quench water
F = The dilution factor
D_3 = The DO content of initial (before incubation) quench water
D_4 = The DO content of final (after incubation) quench water
P_1 = The decimal fraction of dilution water used in the BOD analysis of the quench water

METHOD EVALUATION

Precision

After analyzing a number of quench water samples in duplicate (three final DO determinations were performed to ensure reasonable duplicate results), the precision of the observations were evaluated by calculating (with the Olivetti Programma 101) the pooled standard deviation of all observations except those obtained on samples collected from dump truck drainage.

The results of these calculations are shown in Tables 1 and 2.

TABLE 1
PRECISION OF THE DO ANALYSIS

Type of sample	No. of determinations*	Pooled standard deviation (s)†	Confidence interval ± (1.96)$\sqrt{(2)}$ (s)
Standards (normality)	44	0.13	±0.36‡
Dilution water (blank)	32	0.19	±0.53
Quench water	76	0.49	±1.35
Both dilution and quench water	108	0.43	±1.19

* Includes initial and final determinations.

† A pooled standard deviation was computed for all determinations. It was assumed that there was no statistically significant difference between initial and final variances, that is, homogeneity of the variances was assumed.

‡ The absolute value of the difference between duplicate readings should not exceed 1.96 $\sqrt{2(s)}$, or 0.36 ppm, more than 5 percent of the time. The covariance between the duplicate readings was ignored.

TABLE 2
PRECISION OF THE BOD ANALYSIS

Type of sample	No. of determinations	Standard deviations (S)*	Dilution Factor†	Confidence interval ± (1.96) (40) (S)‡
Dilution water (blank)	8	0.27	40	±21.2
Quench water	19	0.69	40	±54.1
Both dilution and quench water	27	0.61	40	±47.8

* The standard deviation of the difference between initial and final DO readings, (i.e., $S = \sqrt{s^2 \ s^2}$). In this calculation it was assumed that the initial and final pooled variances were equal, and the covariance term between initial and final readings was ignored.

† Dilution factor may vary, but for calculation purposes, the normal dilution factor is shown here.

‡ Ninety-five-percent confidence limits about a single BOD result, assuming a standard dilution factor of 40 or 2.5 percent dilution.

Accuracy

There is no standard against which the accuracy of the BOD test can be measured.

Sensitivity

This Alsterberg (Azide) Modification of the Winkler Method is not applicable to samples that have a dilution factor of 40 and a 5-day BOD value of 54.1 ppm or less.

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APPENDIX

QUALITATIVE TESTS FOR DETERMINING THE PRESENCE OF INTERFERING SUBSTANCES IN QUENCH WATER

INTRODUCTION

Principle of Tests

These qualitative tests may be employed as a quick means of detecting the presence of oxidizing or reducing substances (sulfites, nitrites, ferrous and ferric salts, and residual chlorine for example) that will interfere with the Modified Winkler method for the determination of BOD (Figure 1).

Although the presence of sulfate does not affect the Modified Winkler method, the presence of large quantities of it may indicate the possible presence of sulfite, which definitely affects the method. Likewise, large amounts of chlorides may indirectly affect the method by releasing chlorine gas during acidification.

The principle of each of these tests is discussed briefly in the following sections.

Test for Group A substances (sulfates).

When a barium chloride solution is added to a solution containing a sulfate-type material, an insoluble white precipitate such as barium sulfate will form. This precipitate can be detected visually.

Test for Group B substances (thiosulfates, sulfites).

Thiosulfate will react with mineral acids to yield insoluble elemental sulfur.

$$S_2O_3^{-2} + 2H^+ \rightarrow SO_2 + \underline{S} + H_2O$$

The presence of thiosulfates can be detected when a pale, whitish-yellow precipitate forms after the addition of acid.

In the presence of an acid, dichromate will oxidize iodide to iodine, which will then impart a blue color to the solution when Thyodene is present.

$$Cr_2O_7^{-2} + 6I^- + 14H^+ \rightarrow 2Cr^{+3} + 3I_2 + 7H_2O$$

$$I_2 + \text{Thyodene} \rightarrow \text{Blue complex}$$

Sulfite-like material may be detected by first treating a sample with an acid-iodide solution and a small amount of Thyodene powder, and then performing a dropper titration using potassium dichromate solution. If sulfite-like material is present in the sample, the volume of dichromate solution required to obtain first a blue solution and then a yellow dichromate color will exceed that required by a reagent blank solution.

Small amounts of sulfite can be detected in the presence of any amount of thiosulfate because, on oxidizing a sulfite-thiosulfate mixture with iodine (neutral solution), the following reaction occurs:

$$SO_3^{-2} + I_2 + H_2O \rightarrow SO_4^{-2} + 2I^- + 2H^+$$

$$2S_2O_3^{-2} + I_2 \rightarrow S_4O_6^{-2} + 2I^-$$

Hydrogen ions are formed only in the case of sulfite. The resulting acidity can be revealed by litmus paper.

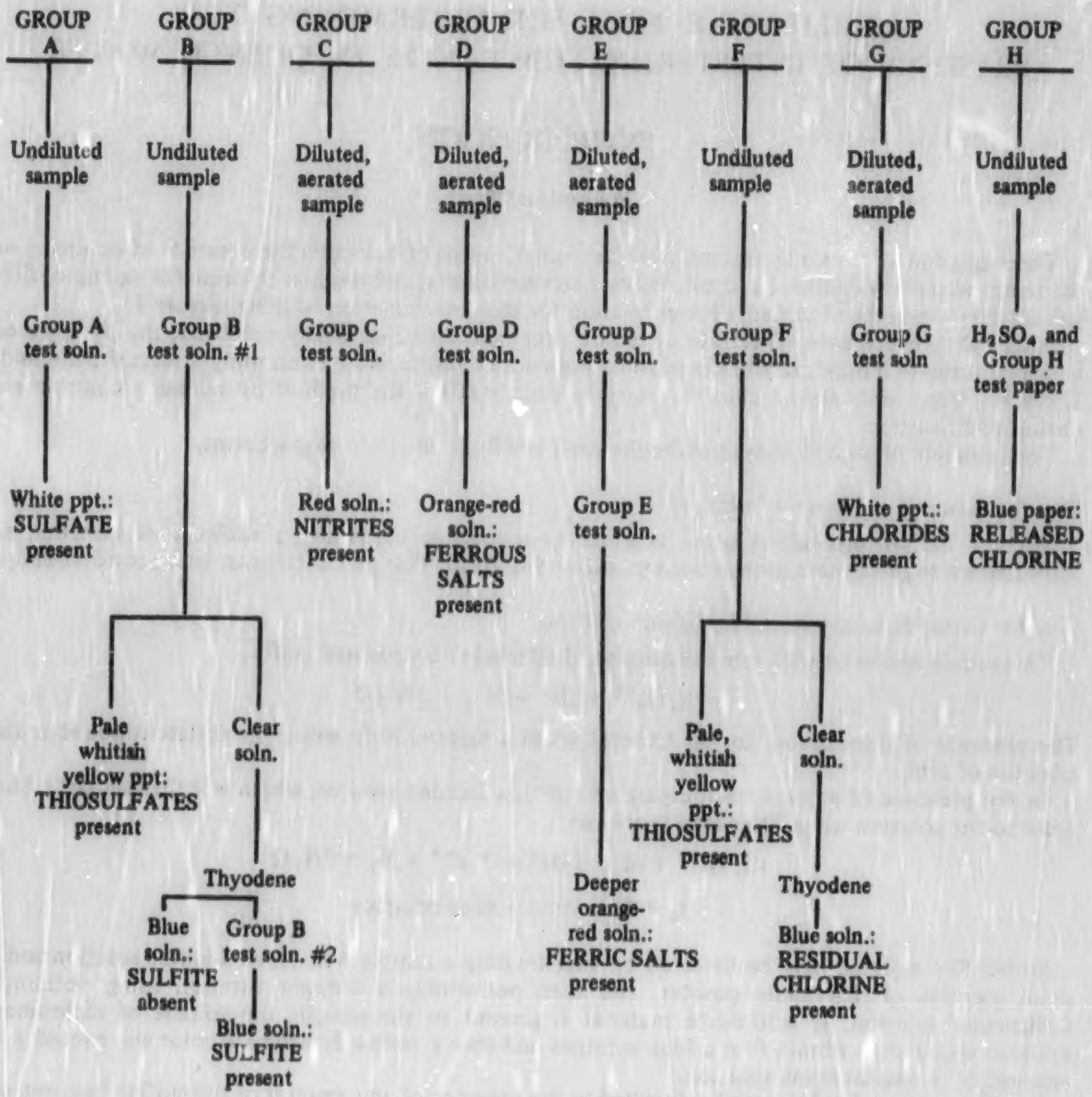

GROUP A
Undiluted sample
Group A test soln.
White ppt.: SULFATE present
GROUP B
Undiluted sample
Group B test soln. #1
Pale whitish yellow ppt: THIOSULFATES present
Clear soln.
Thyodene
Blue soln.: SULFITE absent
Group B test soln. #2
Blue soln.: SULFITE present
GROUP C
Diluted, aerated sample
Group C test soln.
Red soln.: NITRITES present
GROUP D
Diluted, aerated sample
Group D test soln.
Orange-red soln.: FERROUS SALTS present
GROUP E
Diluted, aerated sample
Group D test soln.
Group E test soln.
Deeper orange-red soln.: FERRIC SALTS present
GROUP F
Undiluted sample
Group F test soln.
Pale, whitish yellow ppt.: THIOSULFATES present
Clear soln.
Thyodene
Blue soln.: RESIDUAL CHLORINE present
GROUP G
Diluted, aerated sample
Group G test soln
White ppt.: CHLORIDES present
GROUP H
Undiluted sample
H₂SO₄ and Group H test paper
Blue paper: RELEASED CHLORINE

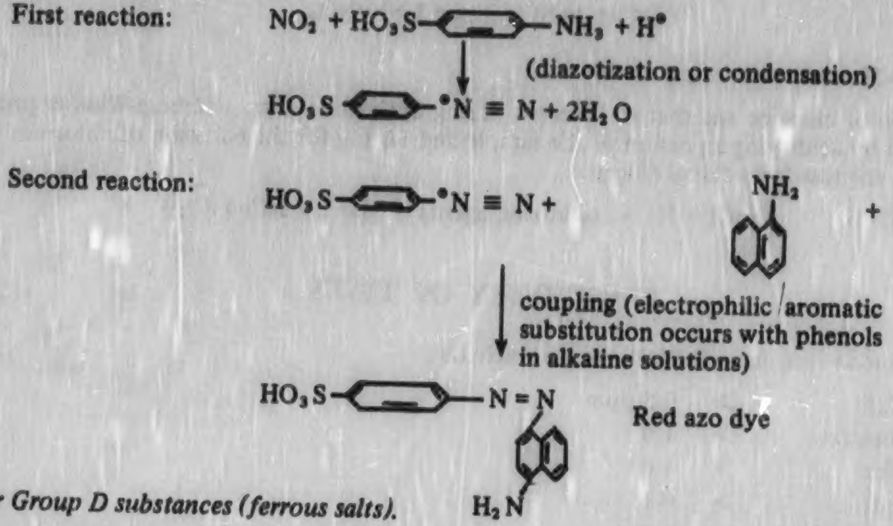
Figure 1. Flow chart of tests for Group A through H substances.

]

The presence of sulfite-type substances in a sample can produce false negative results with the tests for interfering substances that are similar in effect to nitrite, ferrous iron, ferric iron, and residual chlorine. Since, however, no amount of sulfite-type material can be present in a sample, a positive test for sulfite-type compounds eliminates the need for performing the other tests for interferences.

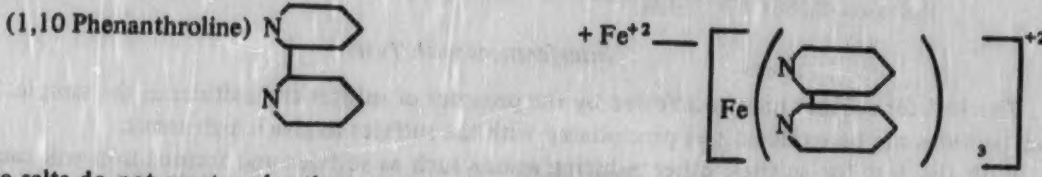
Test for Group C substances (nitrites).

The primary aromatic amine, sulfanilic acid, reacts in an acid solution with nitrites to form diazonium cations that subsequently couple with 1-naphthylamine (1-napthylamine hydrochloride or N-(1-naphtyl)-ethylenediamine dihydrochloride may be substituted) to form the red p-benzene sulfonic acid-azo-α-naphthylamine. The red color is then indicative of the presence of nitrite-type substances.


First reaction:
NO₂ + HO₃S–C₆H₄–NH₂ + H⁺
(diazotization or condensation)
HO₃S–C₆H₄–⁺N ≡ N + 2H₂O
Second reaction:
HO₃S–C₆H₄–⁺N ≡ N +
NH₂
+ H⁺
coupling (electrophilic aromatic substitution occurs with phenols in alkaline solutions)
HO₃S–C₆H₄–N = N
Red azo dye
H₂N

Test for Group D substances (ferrous salts).

In the presence of an acid solution, ferrous salts will react with 1,10 phenanthroline to form a pale orange-red complex; sometimes, however, the sample may have to be filtered to detect the color. More expensive 1,10 dipyridyl can be substituted for the 1,10 phenanthroline. The organic base 1,10 dipyridyl reacts with ferrous iron to form a deep red, very stable, complex cation.


(1,10 Phenanthroline)
N
N
+ Fe⁺²
Fe
N
N
3
+2

Ferric salts do not react under these conditions; consequently, very small amounts of ferrous salts can be detected in the presence of large proportions of ferric salts.

Test for Group E substances (ferric salts).

Ferric salts are reduced by hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) to ferrous salts and allowed to react with 1,10 phenanthroline in the test for Group D. A more intense orange-red color than is found in the test for Group D indicates the presence of ferric-type substances.

Test for Group F substances (residual chlorine).

The presence of chlorine can be detected indirectly by its oxidation of iodide to iodine and the subsequent formation of a blue color in the presence of Thyodene.

$$2I^- + 2H^+ + Cl_2 \rightarrow I_2 + 2HCl$$

$$I_2 + \text{Thyodene} \rightarrow \text{Blue complex}$$

Test for Group G substances (chlorides).

The presence of chlorides can be detected by the addition of silver nitrate to a portion of the sample solution. If chloride-type substances are present, a white precipitate will form.

$$NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$$

Test for Group H substances (released chlorine).

The presence of chlorine gas that is liberated during analysis by the Alsterberg-Winkler procedure can be detected by acidifying a portion of the sample and testing for the emission of chlorine-type gas as described in the test for residual chlorine.

$$3NaCl + H^+ + (\text{oxidizing agent}) \rightarrow Na^{+2} + HCl + \overline{Cl_2}$$

SENSITIVITY OF TESTS

These qualitative tests have the following sensitivities:

Sulfate	>	0.5 ppm
Thiosulfate	>	1.0
Sulfite	>	1.0
Nitrite	>	0.1
Ferrous salt	>	1.0
Ferric salt	>	1.0
Residual chlorine	>	1.0
Chloride	>	1.0
Released chlorine	>	1.0

Interferences with Tests

The test for sulfates may be affected by the presence of sulfites and sulfides in the sample. Sulfites and sulfides can be oxidized and precipitated with the sulfates to give a high result.

With the test for sulfites, other reducing agents such as sulfides and ferrous iron will cause high results. Upon acidification, nitrite in the sample will oxidize all or part of the sulfite. Proteins in the sample will tend to prevent the starch-iodine reaction, and cyanides will react with the iodine in the sample.

The test for nitrites is affected by copper, which catalyzes the deomposition of the diazonium salt. The azo dye reacts with peroxides to form azoxy compounds, with sodium hydroxide to form hydrazo compounds, and with tin chloride to form amines. Also, chlorine bleaches the azo dye, thus causing low results.

Copper, chromium, nickel, and zinc form complexes with 1,10 phenanthroline and thus consume the reagent. Excess amounts of the 1,10 phenanthroline (about 10 times that of the iron concentration) may therefore have to be used for the ferrous (or ferric) salts test.

The tests for residual and released chlorine are not specific because other halogens are also detected. Even ferric iron may oxidize the iodide ions in the test.

The test for chloride is not specific either, since bromide, iodide, and sulfice may precipitate with the chloride to give a high result.

APPARATUS

All glassware must be thoroughly cleaned to ensure the removal of interfering substances. Detergents may be used if cleaning is followed by thorough rinsing with distilled water.

The apparatus requirements are as follows:

1. Test tubes, 16 x 150 mm
2. Test tube rack
3. Reagent bottles, two 100-ml, one 200-ml, two 250-ml, five 500-ml (one of which is a dark bottle), one 1,000-ml
4. Dropper bottles
5. Volumetric flasks, one 100-ml, two 200-ml, one 250-ml, two 500-ml, seven 1,000-ml
6. Graduates, 10-ml, 100-ml, and 500-ml
7. Balances, trip or triple beam and analytical
8. Filter paper
9. Pipets, five 1-ml, one 5-ml, one 50-ml

REAGENTS

Introduction

Since these preliminary tests are qualitative in nature, the concentrations of all reagents except the standard solutions are approximate. The analyst should prepare the standards with care, however, as they serve to familiarize him with the hue and density of color produced by the limit concentration of impurities.

Chemical Requirements

The following chemicals are ACS, reagent grade:

1. Barium chloride
2. Sodium sulfite
3. Concentrated hydrochloric acid
4. Potassium iodide
5. Potassium dichromate
6. Thyodene
7. Sodium nitrite
8. Acetic acid, glacial

9. Sulfanilic acid
10. 1-Naphthylamine (or 1-naphthylamine hydrochloride or N-(1-naphthy) ethylenediamine dihydrochloride)
11. Ferrous sulfate, crystalline
12. 1,10 Phenanthroline (or 1,10 dipyridyl)
13. Ferric chloride, lump
14. Hydroxylamine hydrochloride
15. Iodine
16. Concentrated sulfuric acid
17. Sodium chloride
18. Silver nitrate

Preparation of Solutions

All solutions are prepared with distilled water that (a) was distilled from a block-tin or all-glass still, (b) contains less than 0.01 mg per liter copper, and (c) is free of chlorine, chloramines, caustic alkalinity, organic materials, and acids. The solutions are prepared in the following manner:

1. Group A—standard stock solution: Dissolve 0.3697 g Na_2SO_4 in a 1,000-ml flask with distilled water and dilute to the mark. This stock solution contains 250 ppm sulfate ion and is stable for at least 1 week.
2. Group A—standard working solution: Pipet 1 ml of stock solution into a 500-ml flask and dilute to the mark. This working solution contains 0.5 ppm sulfate ion and should be prepared the same day it is to be used.
3. Group A—test solution: Dissolve 5 g $BaCl_2$ in a 500-ml reagent bottle with distilled water and dilute to about 500 ml. This solution is stable for at least 1 week.
4. Group B—standard stock solution: Dissolve 0.3936 g Na_2SO_3 in a 1,000-ml flask with distilled water and dilute to the mark. This stock solution contains 250 ppm sulfite ion and should be prepared the same day it is to be used.
5. Group B—standard working solution: Pipet 1 ml of stock solution into a 250-ml flask and dilute to the mark. This working solution contains 1.0 ppm sulfite ion and should be prepared the same day it is to be used.
6. Group B—test solution No. 1: Prepare daily by dissolving 2 g KI in a 250-ml reagent bottle with about 100 ml of distilled water. Add 10 ml conc. HCl and dilute to about 250 ml.
7. Group B—test solution No. 2: Dissolve 2 g $K_2Cr_2O_7$ in a 500-ml reagent bottle with distilled water and dilute to about 500 ml. This solution is stable for about 1 week.
8. Group C—standard stock solution: Dissolve 0.6000 g $NaNO_2$ in a 1,000-ml flask with distilled water and dilute to the mark. This stock solution contains 400 ppm nitrite ion and should be prepared the same day it is to be used; if stored in a refrigerator, however, the stock solution is stable for 1 week.
9. Group C—standard working solution: Pipet 1 ml of stock solution into a 100-ml flask and dilute to the mark. This working solution contains 4.0 ppm nitrite ion and should be prepared the same day it is to be used.
10. Group C—test solution: Dissolve 10 ml acetic acid, 10 g sulfanilic acid, and 1 g 1-naphthylamine (or 1-naphthylamine hydrochloride or N-(1-naphthy)ethylenediamine dihydrochloride) in a 1,000-ml reagent bottle with distilled water and dilute to about 1,000 ml. Prepare daily.
11. Group D—standard stock solution: Dissolve 0.9955 g $FeSO_4 \cdot 7H_2O$ in a 1,000-ml flask with

distilled water and dilute to the mark. This stock solution contains 200 ppm ferrous iron and should be prepared the same day it is to be used.

12. Group D–standard working solution: Pipet 1 ml of stock solution into a 200-ml flask and dilute to the mark. This working solution contains 1 ppm ferrous iron and should be prepared each day it is to be used.
13. Group D–test solution: Dissolve 5 g 1,10 phenanthroline (or 1,10 dipyridyl) in a 500-ml reagent bottle with distilled water and dilute to about 500 ml. Prepare daily.
14. Group E–standard solution No. 1: Dissolve 0.9676 g $FeCl_3 \cdot 6H_2O$ in a 1,000-ml flask with distilled water and dilute to the mark. This No. 1 standard solution contains 200 ppm ferric iron. Prepare daily.
15. Group E–standard solution No. 2: Pipet 50 ml of the above No. 1 standard solution into a 200-ml flask and dilute to the mark with distilled water. This No. 2 standard solution contains 50 ppm ferric iron. Prepare daily.
16. Group E–test solution: Dissolve 5 g hydroxylamine hydrochloride in a 500-ml reagent bottle with distilled water and dilute to about 500 ml. Prepare about once each week.
17. Group F–standard solution: Dissolve about 1 g iodine in distilled water and dilute to 1,000 ml. Prepare about once each week.
18. Group F–test solution: Prepare daily by dissolving 2 g KI in a 250-ml reagent bottle with about 100 ml of distilled water. Add 5 ml conc. H_2SO_4 and dilute to about 250 ml.
19. Group G–standard stock solution: Dissolve 0.8243 g NaCl in a 500-ml flask with distilled water and dilute to mark. This stock solution contains 500 ppm chloride ion and is stable for about 1 week.
20. Group G–standard working solution: Pipet 1 ml of stock solution into a 500-ml flask and dilute to the mark. This working solution contains 1.0 ppm chloride ion and should be prepared the same day it is to be used.
21. Group G–test solution: Dissolve 5 g $AgNO_3$ in a 500-ml, dark reagent bottle with distilled water and dilute to about 500 ml. Prepare about once each week.
22. Group H–standard solution: Either the standard solution of Group F or the standard stock solution of Group G may be used.
23. Group H–indicator solution: Dissolve 5 g Thyodene in a 100-ml reagent bottle with distilled water and dilute to about 100 ml. This solution is stable for several weeks.
24. Group H–test paper: Impregnate 1/4-in.-wide strips of ordinary filter paper with Group F test solution and indicator solution immediately before use.

STANDARDIZATION

No special standardization is needed. The standard solutions mentioned under "Reagents" are analyzed along with the unknown samples, however. This allows the analyst to compare the color of an unknown to the color intensity of the known impurity at maximum tolerable concentration.

SAMPLE ANALYSIS

Test for Group A substances (sulfates).

Procedure	Comments
1. Pour equal volumes of sample (or standard working solution) and test solution into test tube.	1. A cloudy white solution indicates Group A and/or Group B.

Test for Group B substances (thiosulfates, sulfites).

Procedure	Comments
1. Pour equal volumes of sample and test solution No. 1 into test tube. If sample turns a cloudy, whitish yellow, stop at this point. If sample remains clear, go on to step 2.	1. a) Use sulfite standard working solution as a guide. b) A cloudy, whitish yellow indicates thiosulfates or the like.
2. Add a small amount of Thyodene powder to this clear mixture and to another test tube with an equal volume of test solution No. 1. If sample solution remains clear, go to step 3.	2. a) Test solution No. 1 may turn pale blue because of some iodine created during preparation of the solution. b) If mixture with sample turns pale blue, sulfite is not present.
3. Perform a dropper titration with each solution, using test solution No. 2 until each solution has the yellow color of the test solution No. 2.	3. a) The test solution No. 1 should turn yellow with only 1 to 2 drops of titrant. b) If the sample solution turns blue with 1 to 2 drops of titrant, and yellow after several drops, sulfite-type substances are present. The amount of titrant used for the sample mixture must exceed the amount needed for test solution No. 1 for sulfite-type substances to be present.
4. If only a slight amount of sulfite-type substance is present, repeat test with aerated, diluted sample.	4. Sulfite-type substances may react and disappear upon aeration.

Test for Group C substances (nitrites).

Procedure	Comments
1. Pour equal volumes of the diluted, aerated sample and test solution into test tube.	1. a) Test may first be performed with undiluted sample. b) Use dilution employed in DO determination.
2. After a few minutes, look for the red color of an azo dye.	2. a) Reagent reference blank may be needed. b) Use nitrite standard working solution as a guide for elimination of nitrite concentration. c) If Group B is present, this test shows a negative result.

Test for Group D substances (ferrous salts).

Procedure	Comments
1. Pour equal volumes of diluted, aerated sample and test solution into test tube.	1. a) Test may first be performed with undiluted sample. b) Use dilution employed in DO determination. c) Use Group D standard working solution as a guide.

2. After a few minutes, inspect the solution for the formation of an orange-red color, indicative of the presence of ferrous salts.

2. a) Sample may need filtering to see to color.
   b) If Group B is present, this test shows a negative result.
   c) The interference from nitrite ion may be eliminated by adding an excess of test solution.

Test for Group E substances (ferric salts).

Procedure

1. Treat a portion of sample (standard solutions No. 1 and No. 2 and diluted, aerated or non-diluted quench water) as directed in steps 1 and 2 of test for Group D substances.
2. Add to each test tube an equal volume of test solution.
3. After a few minutes, inspect the solution for the formation of an orange-red color that is deeper than the color formed in the Group D test.

Comments

1. May use same sample portion employed in the test for Group D substances.

3. a) Comments for step 2 of the Group D test apply here also.
   b) If the intensity of the color is greater than standard solution No. 1 but less than No. 2, the Modified Winkler Method can be employed, provided the potassium fluoride is used.

Test for Group F substances (residual chlorine).

Procedure

1. Pour equal volumes of undiluted sample (or standard) and test solution into a test tube. If the sample mixture turns a cloudy, whitish yellow (as in Group B test), stop at this point. If the solution is clear, go to step 2.
2. To the clear sample and reagent blank add a small amount of Thyodene powder.
3. If a blue color forms that is deeper than the blank, a chlorine-like substance is present.
4. If the test is positive, repeat with a diluted, aerated sample.

Comments

1. a) More readily available iodine may be used as a standard instead of chlorine.
   b) Use a reagent (test solution) blank.
   c) Cloudy, whitish yellow is indicative of thiosulfate.
2. Test solution may turn pale blue because of some iodine created during preparation of the solution.

4. Chlorine-like substances may dissipate upon aeration.

Test for Group G substances (chlorides).

Procedure

1. Pour equal volumes of the diluted, aerated sample (or standard) and test solution into test tube.

Comments

1. Cloudy white solution indicates Group G. A heavy white precipitation indicates possible interference from Group H substances.

Test for Group H substances (released chlorine).

Procedure	Comments
1. Fill half a test tube with sample (or standard).	1. This test need only be performed if the test sample showed no Group F substances and high amounts of Group G substances.
2. Pour 1 to 2 ml of concentrated H_2SO_4 into a test tube.	
3. Immediately place test paper into upper half of test tube.	
4. After a few minutes, look for the paper to turn blue.	4. Blue color indicates a free halogen, which interferes with the Modified Winkler method.

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THE DISSOLVED OXYGEN ANALYZER (WESTON & STACK, INC., MODEL 300-B) METHOD FOR DETERMINING THE BOD OF INCINERATOR QUENCH WATER

Donald L. Wilson*

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INTRODUCTION

The analysis of an aerated, diluted sample for its BOD involves the determination of its dissolved oxygen (DO) content before and after an incubation period. The difference between the initial DO and the final oxygen content represents the oxygen demand of the sample.

The oxygen demand of incinerator quench water* (or similarly polluted water) is exerted by three classes of materials: (a) carbonaceous organic material usable as a food source by aerobic organisms; (b) oxidizable nitrogen derived from nitrite, ammonia, and organic nitrogen compounds that serve as food for specific bacteria (e.g., Nitrosomonas and Nitrobacter); and (c) certain chemical reducing compounds (e.g., ferrous iron, sulfite, and sulfide) that will react with molecularly dissolved oxygen. Since the oxidation of nitrogenous materials may proceed at a variable rate, the nitrification process is inhibited, thus restricting the BOD determination to the organic carbon present. The water sample is acidified to pH 2 to 3 and subsequently neutralized to accomplish the inhibition of the nitrification.

Complete stabilization of a given sample may require an overly long incubation period for practical purposes. The 5-day incubation period has been accepted as standard. For certain industrial wastes, however, it may be advisable to determine the oxidation curve. Conversion of data from one incubation period to another can only be made if such special studies are carried out. Studies in recent years have shown that the exponential rate of carbonaceous oxidation at 20 C rarely has a value of 0.1, but may vary from less than one-half to more than twice this value. This fact usually makes it impossible to calculate the ultimate carbonaceous demand of a sample from 5-day BOD values, unless the exponential-rate value has been determined on the contaminated water under consideration.

Since incinerator quench water may contain many variables that affect the Winkler Method of analysis, the Dissolved Oxygen Analyzer Method is recommended for BOD analysis of all quench water samples. The Alsterberg (Azide) Modification of the Winkler Method is recommended for standardization of the Analyzer using the relatively pure dilution water. Preliminary tests, which show the validity of the Winkler DO value, are discussed in the chapter on the Winkler Method.

The sampling location at each site is very important in the evaluation of the data and should be chosen on the basis of obtaining the most representative sample.

DISCUSSION

The Weston & Stack Dissolved Oxygen (DO) analyzer uses a specially designed probe to measure accurately and quickly the amount of dissolved oxygen in gas streams and liquids. The probe is constructed of cast epoxy and is separated from the sample by a semipermeable membrane.

The analyzer is powered by AC or internal batteries and is ruggedly constructed and moisture-proof to facilitate laboratory or field use. Interferences in water or gas samples are minimal with this instrument. Hydrogen sulfide does not interfere, but will eventually corrode the lead anode. The probe will then require cleaning. Dissolved or suspended solids will not affect the probe, provided the analyzer is calibrated using a similar type of sample to account for partial pressure changes. Laboratory tests have reaffirmed that this probe is not affected by ferrous or ferric iron, sulfite, or nitrite.

Since temperature affects the rate of diffusion of dissolved oxygen through the Teflon membrane, the probe output for a given concentration of dissolved oxygen is a function of the temperature. A secondary resistance to oxygen diffusion exists at the Teflon-aqueous sample interface. The interfacial resistance is of minor significance when the sample is vigorously agitated to produce a high degree of turbulence, a condition that is also necessary for the temperature compensation to function satisfactorily.

Temperature compensation in the Weston & Stack analyzer is accomplished by an operational amplifier. A thermistor (a resistor whose resistivity varies intensely with temperature) and a resistance network introduced into the feedback circuit for the amplifier provide suitable multiplication so that temperature effect on the probe is limited to ± 2 percent over the temperature range of 0 to 50 C when suitable turbulence is provided.

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*Quench water refers to water that has been used to cool the non-combustibles after emergence from the furnace.

The probe must be calibrated by the Winkler Method to enable the analyst to read the true ppm DO directly from the scale of the instrument. This calibration, although normally needed only once a month, preferably should be checked at the beginning and end of each 5-day incubation period.

Although a probe output can be obtained for any element or compound that diffuses through the semipermeable (Teflon) membrane and is reduced at a potential of –0.578 volts or less, interferences of this nature appear to be infrequent. Sulfite, nitrite, ferrous and ferric iron, and other reducing and oxidizing substances that normally interfere with the Winkler Method apparently do not affect the output of this probe. There are a few substances, however, that do affect the sensitivity of the probe over a period of time. Hydrogen sulfide and chlorine, although not detected by the probe, will react with the lead anode and cause a decline in sensitivity. Greases and oils will coat the semipermeable membrane, increase the diffusion resistance, and decrease the probe output. Variations in dissolved solids will alter the partial pressure of oxygen in the aqueous samples and hence the probe output. The calibration and use of the instrument should be accomplished with these facts in mind.

APPARATUS

Requirements

1. Analyzer, Weston & Stack, Model 300-B, DO Ranges: 0-1.5 ppm and 0-15 ppm, temperature compensation and temperature readout; AC powered with internal combination power-supply and battery-charger
2. Probe, Weston & Stack, Model A-30 BOD agitator-thermistor assembly
3. Accessory kit containing membranes, electrolyte, syringe, manual, recorder, and plug
4. Extra membranes, Teflon, ½ mm thick, 3 in. sq, 24 per package
5. Graduates, 50-ml, 1-liter, and 2-liter
6. Beakers, 250-ml, 2-liter, and 3-liter
7. Siphon tubing
8. Rubberbands
9. BOD bottles, 300-ml capacity
10. Analytical balance
11. Volumetric flasks, 100-ml, 500-ml, and five 1-liter
12. pH paper, range 2 through 9
13. Air incubator or waterbath, thermostatically controlled at 20 C ± 1 C
14. Carboy, polyethylene nalgene, wide-mouth, two 2-gal (7 liters or more)
15. Test tube with 14 mm I.D.
16. Magnetic stirrer with Teflon-coated stirring bar
17. Stopwatch or accurate watch with second hand
18. Pipets, graduated or volumetric, two 2-ml
19. Reagent bottles, resistant glass, narrow-mouth with ground glass stoppers, 1-liter capacity, at least one amber colored
20. Sample collection bottles, polyethylene (or similar unbreakable bottle) with narrow mouth and tightly fitting caps, about 1-liter (or 1-qt) capacity, sterile

21. Regulator, 2-stage, with cylinder valve outlet for nitrogen (CGA No. 580)
22. Ice chest, capable of holding several 1-liter sample collection bottles and maintaining a 5 C temperature for 24 hr.

Preparation and Maintenance

Probe.

Membrane Installation. Since the performance of the probe is dependent upon a properly installed membrane, the analyst should exercise great care in performing the following instructions.

Procedure	Comments
1. Remove the probe shield and thermistor housing, electrolyte fill screw, and old membrane from the probe.	1. See Figure 1 for identification of probe components.
2. Wrap a rubberband around a test tube, having a 14-mm I.D.	2. See Figure 2 (a-f) for the diagrammatic representation of steps 2, 6, 7, 9, 10 and 12.
3. Hold the test tube in an upright position by encircling it with the fingers and thumb of one hand.	3. The open end of the test tube should be flush with the forefinger.
4. Place one membrane sheet over the top of the test tube and fist.	
5. Gently depress the membrane about 1 in. into the tube.	
6. Pour electrolyte into the depression.	6. See preparation of electrolyte.
7. Press the membrane down into the tube with the probe.	7. Do this carefully so the membrane is not damaged. Keep air bubbles from being trapped between the probe and membrane.
8. Slip the rubber band off the tube and over the membrane to a point ½ in. above the holes near the tip of the probe.	8. Remove the tube.
9. Carefully draw up the membrane to provide a snug fit.	9. It is necessary to have a close, smooth fit over the platinum electrode without stretching or tearing the membrane.
10. Wrap a second rubber band as tightly as possible just above the holes.	10. Apply securely to prevent leakage of electrolyte from membrane.
11. Remove the first (top) rubber band.	
12. Trim membrane close to and above the second rubber band.	
13. Use the syringe to completely fill the cavity in the probe with electrolyte.	13. The probe should be held upright.
14. Shake and tap the probe so that air bubbles will escape from the fill hole.	14. Thumb is held over fill hole during shaking.
15. Replace the fill screw and probe shield.	
16. Connect the probe to the readout instrument and turn the meter to "ON" and the selector switch to "DO-MULT 1."	16. See Figure 3 for the location of components of the instrument.

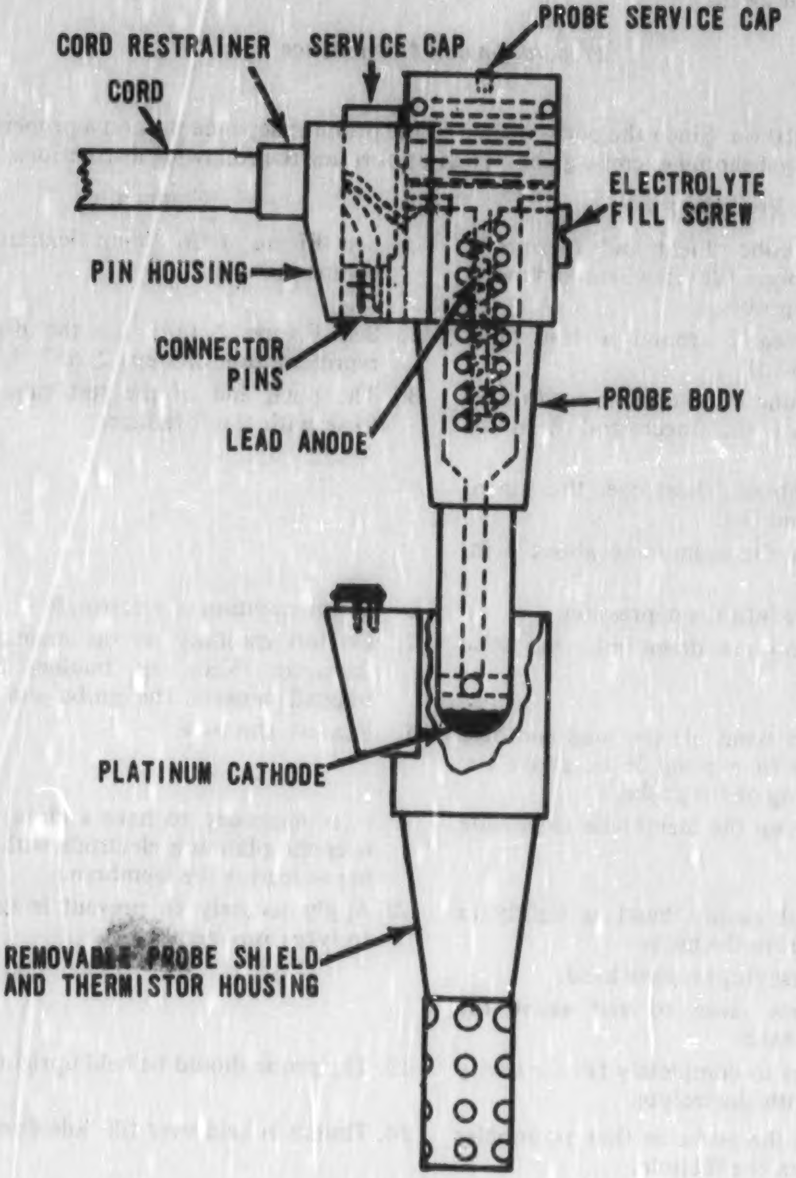
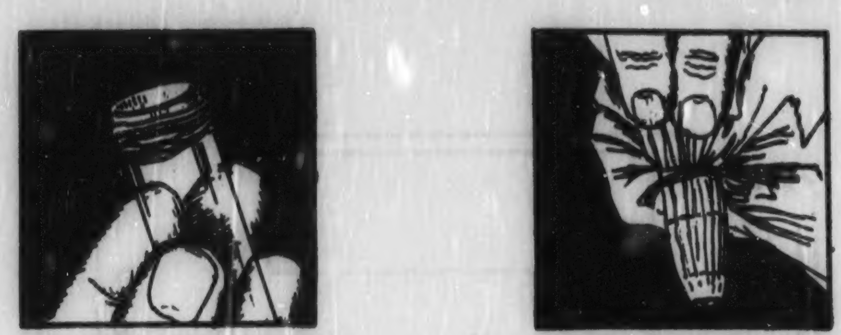
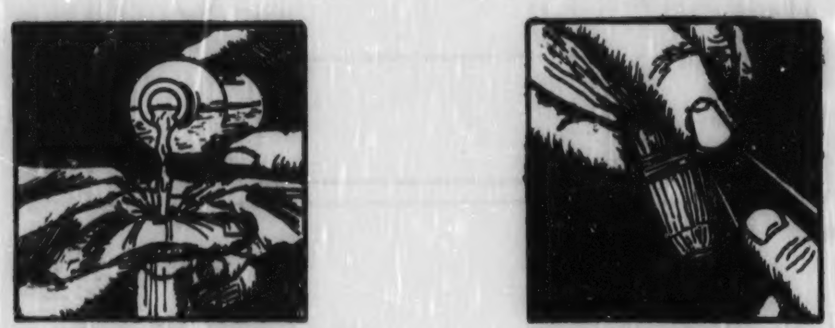

PROBE SERVICE CAP
CORD RESTRAINER
SERVICE CAP
CORD
ELECTROLYTE FILL SCREW
PIN HOUSING
CONNECTOR PINS
PROBE BODY
LEAD ANODE
PLATINUM CATHODE
REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 1. The probe. (Reproduced with permission of Weston & Stack, Inc.)



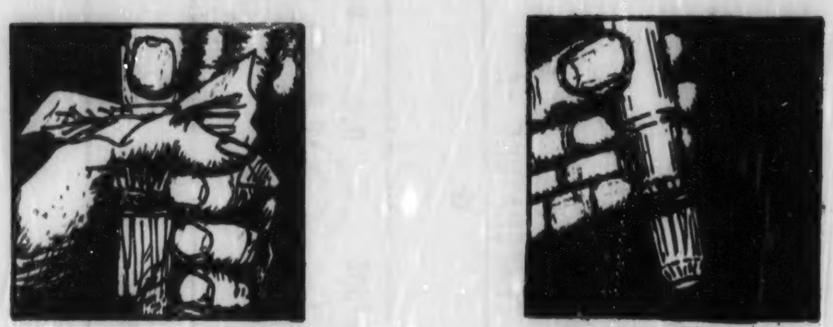
a. Step #2: Position of the rubber band on the test tube.

d. Step #8: Drawing up the membrane to provide a snug fit across the face of the platinum tip.



b. Step #6: Pouring the electrolyte into the depressed membrane.

e. Step #10: Wrapping a second rubber band around the probe just above the small holes.



c. Step #7: Pressing the membrane down into the tube with the probe.

f. Step #12: The probe after the removal of the upper rubber band and the trimming of the membrane.

Figure 2. Membrane installation procedure. (Drawings reproduced with permission of Weston & Stack, Inc.)

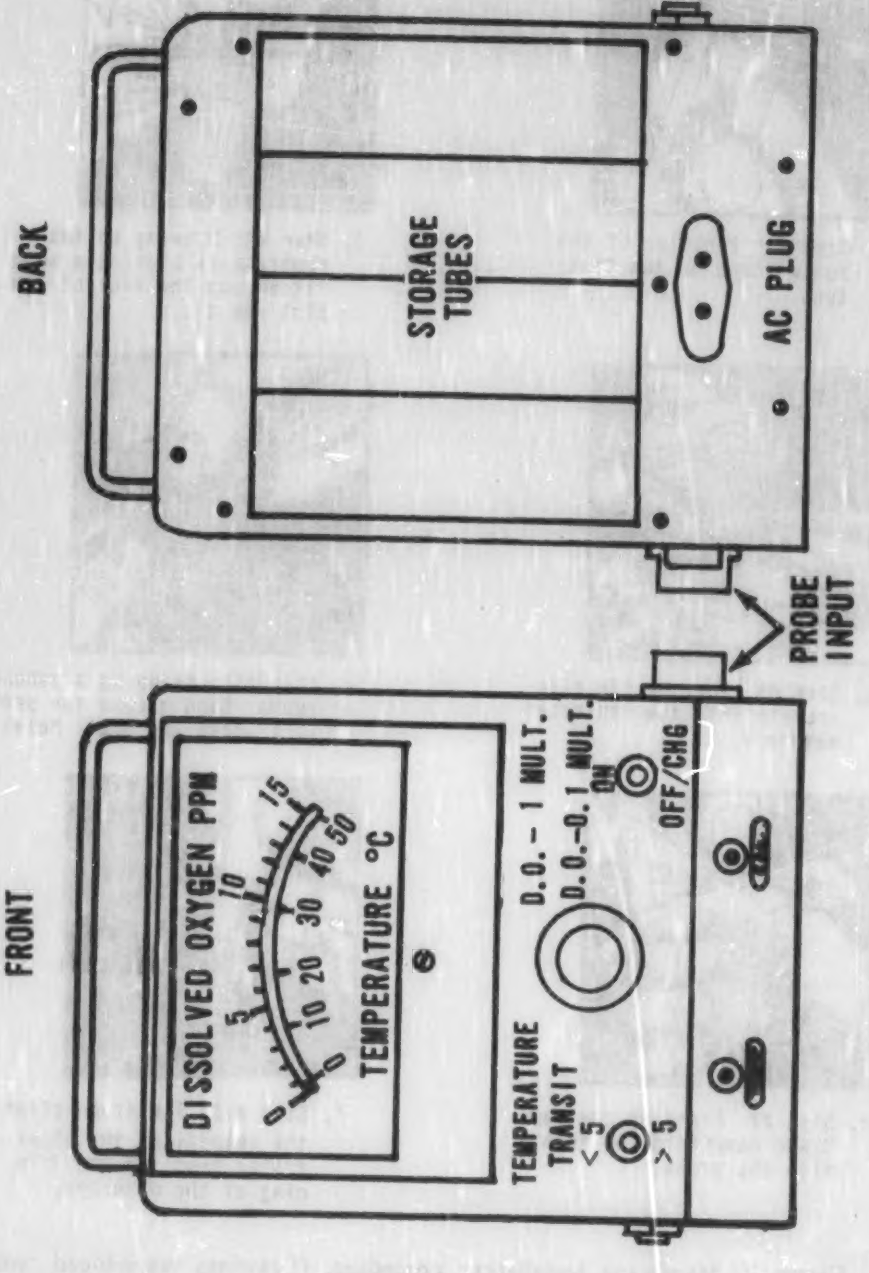

FRONT
BACK
DISSOLVED OXYGEN PPM
0
5
10
15
0
10
20
30
40
50
TEMPERATURE °C
TEMPERATURE
TRANSIT
< 5
> 5
D.O. – 1 MULT.
D.O.–0.1 MULT.
ON
OFF/CHG
STORAGE TUBES
AC PLUG
PROBE INPUT

Figure 3. Front and back views of the Analyzer. (Reproduced with permission Weston & Stack, Inc.)

17. Hold the probe with platinum electrode up and shake it vigorously.	17. If the meter needle oscillates, air bubbles are present and steps 14 through 17 must be repeated.

Detection of Membrane Perforation. When a hole develops in the membrane, the response rate of the probe decreases as the electrolyte is diluted and the cathode is poisoned. To ensure the proper performance of the probe, frequent inspections of the membrane should be performed:

Procedure	Comments
1. Hold the membrane end of the probe in a beaker containing clear water.	1. If the membrane was installed recently, the probe should be rinsed thoroughly to remove electrolyte that may be trapped in the folds of the membrane.
2. While looking through the water towards a light source, search for a small stream (diffused light) of electrolyte floating through a hole in the membrane.	2. If a hole is detected, a new membrane must be installed.

Servicing a Contaminated Probe. After sitting for a few months with electrolyte in it, the inner parts of the probe become contaminated and may not allow any calibration adjustment to be made or cause the readout needle to drift downward. The procedure for cleaning a probe is as follows:

Procedure	Comments
1. Remove the probe shield and thermistor housing, membrane, electrolyte fill screw, and probe service cap screw (may use quarter coin).	1. See Figure 1 for identification of probe parts.
2. Turn probe upside down and shake out the lead anode.	
3. Clean the lead anode by immersing it in warm, 10-percent NaOH (or HCl) solution; then rinse thoroughly with distilled water.	3. All the yellow deposit must be removed.
4. Clean the platinum electrode and the inside of the probe body with 6 N (1:1)HCl, then rinse thoroughly with distilled water.	4. The lead anode will not seat properly or make contact if the lead ring inside the probe is not completely clean.
5. Polish the platinum cathode with a soft tissue.	
6. Reassemble lead anode and probe service cap.	6. Use a small amount of silicon grease on the threads and the "O" ring.
7. Install a new membrane as previously directed.	7. See Membrane Installation.

Glass and plastic apparatus.

Sampling bottles, tubing, containers, and the like must be thoroughly cleaned (sterile) to ensure the removal of materials capable of exerting a BOD. Detergents may be used if cleaning is followed by thorough rinsing with distilled water.

Recharging batteries.

The Weston & Stack Dissolved Oxygen Analyzer, Model 300-B, is provided with an internally combined AC power supply and battery charger. The instrument can be operated in the laboratory directly on 110 AC or in the field, using the rechargeable nickel cadmium batteries. When the instrument is employed in the field, a record of the hours of amplifier usage should be maintained. Before each standardization of the instrument in the laboratory or utilization in the field, the analyst should then check his record. If the amplifier usage ≥ 20 hr, the batteries should be recharged as follows:

Procedure	Comments
1. While operating the instrument on 110 AC, place the selector switch on "DO-Mult 1."	1. See Figure 3 for the location of the components of the instrument.
2. Turn the power switch to "OFF/CHG."	
3. Determine if a charge is coming from the batteries.	3. The meter needle should respond and possibly oscillate. If no response occurs, check the 12-volt batteries and connections. Replace components if necessary.
4. Place selector switch in the "Transit" position and recharge the batteries for a maximum of 10 hr.	4. Rechargeable batteries last about 3 years.

NOTE: The 30-volt battery is not rechargeable and lasts about 6 months.

REAGENTS

Chemical Requirements

The following chemicals are ACS, reagent grade:

1. Potassium iodide
2. Sodium sulfite
3. Sodium hydroxide
4. Hydrochloric acid, concentrated
5. Sulfuric acid, concentrated
6. Phosphate buffer solution, pH 7.2 (or prepared)
7. Potassium phosphate, monobasic
8. Potassium phosphate, dibasic
9. Sodium phosphate, dibasic, heptahedrate, crystalline
10. Ammonium chloride
11. Magnesium sulfate, crystalline
12. Calcium chloride, anhydrous
13. Ferric chloride, lumps
14. Manganese(ous) sulfate, monohydrate
15. Potassium hydroxide
16. Nitrogen, 99.9 percent pure

Preparation of Solutions

The water employed in the preparation of solutions must (a) be distilled from a block-tin or all-glass still, (b) contain less than 0.01 mg copper per liter, and (c) be free of chlorine, chloramines, caustic alkalinity, organic materials, and acids.

Solutions are prepared as follows:

1. Electrolyte solution: Dissolve 50.0 g of potassium iodide in distilled water and dilute with same to 100 ml. Store solution in a dark brown bottle (8-oz bottles of this solution may be purchased from either the Weston & Stack Company, 1426 Lewis Lane, West Chester, Pennsylvania, 19380, or their Ohio representative, Henry P. Thompson Co., 4866 Cooper Road, Cincinnati, Ohio, 45242).
2. Sodium sulfite solution: Dissolve about 5 g of sodium sulfite in 500 ml of distilled water.
3. Sodium hydroxide solution, approximately 1 N: Dissolve 41.6 g NaOH in distilled water and dilute with same to 1 liter.
4. Sulfuric acid solution, approximately 1 N: Cautiously add 28 ml of concentrated H_2SO_4 to distilled water and dilute with same to 1 liter.
5. Dilution Water: To 1 liter of distilled water at 20 C, add 1 ml of each of the following solutions: phosphate buffer solution, pH 7.2; magnesium sulfate solution (22.5 g $MgSO_4 \cdot 7H_2O$ per liter of solution); calcium chloride solution (27.5 g anhydrous $CaCl_2$ per liter of solution); ferric chloride solution (0.25 g $FeCl_3 \cdot 6H_2O$ per liter of solution).
6. Sodium hydroxide solution, 10 percent (w/v): Dissolve 10.0 g NaOH in distilled water and dilute with same to 100 ml.
7. Phosphate buffer solution, pH 7.2, may be purchased in prepared form or may be prepared as follows: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl in 500 ml distilled water and dilute with same to 1 liter. The pH of this buffer should be 7.2 without further adjustment.
8. Hydrochloric acid solution, 10 percent (v/v): Cautiously add 10 ml concentrated HCl (sp. gr. 1.19) to 75 ml distilled water and dilute with the latter to 100 ml.
9. Hydrochloric acid solution, 5 N: Cautiously add 42.8 ml concentrated HCl (sp. gr. 1.19) to 40 ml distilled water and dilute with the latter to 100 ml.
10. Manganous sulfate solution, 0.25 M: Dissolve 21.13 g $MnSO_4 \cdot H_2O$ in distilled water and dilute with same to 500 ml.
11. Potassium hydroxide solution, 0.5 M: Dissolve 14.03 g KOH in distilled water and dilute with same to 500 ml.

SAFETY PRECAUTIONS

Follow general laboratory safety rules. This method has no pronounced safety hazards.

CALIBRATION

Zero Adjustment of Amplifier

To ensure the proper amplification of the temperature-compensated signal from the probe, the output of the amplifier should first be adjusted to zero while the probe is inserted into a solution containing no dissolved oxygen. The procedure is as follows:

Procedure	Comments
1. Set the selector switch to "Transit" and power switch to "OFF/CHG."	1. See Figure 3 for the location of components of the instrument.
2. Using the unmarked screw on the meter face, set the meter needle to zero.	2. Adjustment may not be necessary.
3. Place the probe in the sodium sulfite solution for 2 min or longer.	3. Use a regular BOD bottle to contain the solution.
4. Set the selector switch to "DO-Mult 1" and turn the Analyzer to "ON" for a period of at least 2 min.	4. The agitator should be employed.
5. Adjust the meter reading to zero using the zero-adjustment screw (marked "zero") on the front of the instrument case.	
6. Remove the probe from the sulfite solution and rinse the membrane thoroughly with distilled water.	6. Keep the probe in BOD bottle of clean, distilled water when not in use.

Temperature Compensation of the Probe's Output

A thermistor (a resistor whose resistivity varies intensely with temperature) and a resistance network introduced into the feedback circuit of an operational amplifier provide the temperature compensation of the probe's output. The compensation is accurate to ± 2 percent over a sample temperature range of 0 to 50 C. An adjustment is necessary, however, if sample temperature varies more than 5 C from the temperature of the probe. The adjustment is as follows:

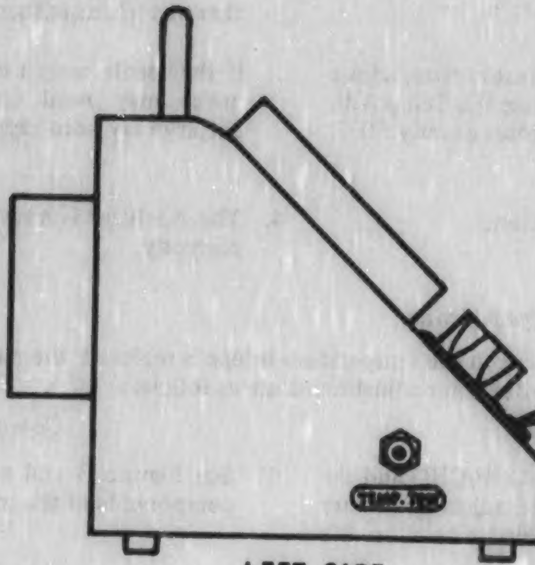
Procedure	Comments
1. If the probe and sample are not essentially the same temperature (within 5 C), move front-left switch (marked >5 & <5) to the >5 position.	1. a) See Figure 3 for the location of the switch. b) Analyzer may be used to check temperatures of room and sample (see Sample Analysis).
2. If the probe and sample are near the same temperature (within 5 C), move above switch to the <5 position.	2. Normally this step is applied.

Temperature Scale

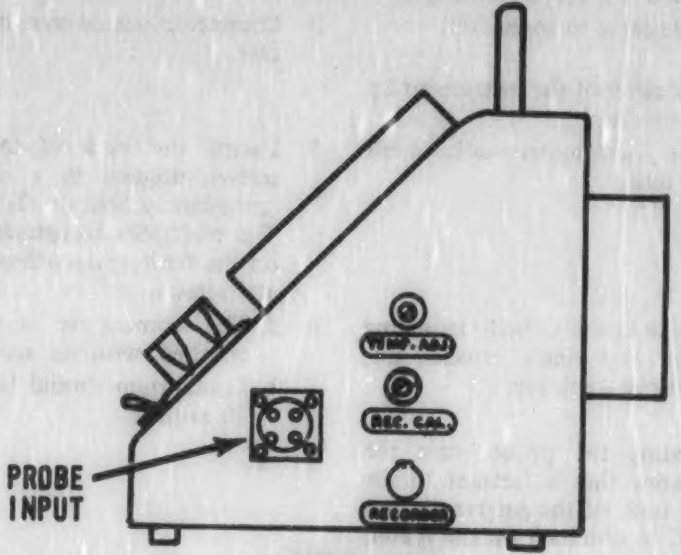
Regular adjustment of the bridge potential.

The temperature scale of the instrument is calibrated at the factory and in normal operation should not require re-calibration. The thermometer circuit is, however, designed as an unbalanced bridge whose potential is supplied by a 30-volt battery. Regular (each day of use) adjustments of the bridge potential should, therefore, be performed as follows:

Procedure	Comments
1. Turn the selector switch to Temperature.	1. See Figures 3 and 4 for the location of the switch and other components of these instructions.



LEFT SIDE


TEMP. ADJ
REC. CAL.
RECORDER
PROBE INPUT

RIGHT SIDE

Figure 4. Side view of Analyzer. (Reproduced with permission of Weston & Stack, Inc.)

2. Press the Temp Test button.	2. The resistor's activity is equivalent to the thermistor's resistance at 50 C.
3. While pressing the Temp Test button, adjust the potentiometer by turning the Temp Adj. screw until the needle indicates exactly 50 C.	3. If the needle cannot be adjusted to 50 C, the probe may need cleaning or the 30-volt battery may need replacement.
4. Release the Temp Test button.	4. The Analyzer is now indicating temperature correctly.

Special adjustment of the bridge potential.

When a resistance component in the temperature bridge is replaced, the temperature scale must be recalibrated. The special potentiometer adjustments are as follows:

Procedure	Comments
1. With the power switch on OFF/CHG and the selector switch on Transit, adjust the meter to zero using the unlabeled screw on the meter face.	1. See Figures 3 and 4 for the location of the components of the instrument.
2. Assemble probe and thermistor housing.	
3. Connect the probe cable to connector.	3. Connector located on right side of instrument case.
4. Remove the back cover of the instrument by removing all eight screws.	
5. Locate the three potentiometer-adjustment screws inside the unit.	5. Facing the back of the unit, four small screws, aligned in a horizontal line, are immediately beneath the handle attachment. The multiplier adjustment screw is the one on the far left; the others are the potentiometer screws.
6. Immerse the probe in a 0 C bath consisting of distilled water and finely crushed ice; then agitate the probe assembly.	6. a) The temperature of the bath should be checked with an accurate thermometer. b) Equilibrium should be reached after 5 to 10 min.
7. While still agitating the probe, turn the potentiometer screw that is farthest to the right (facing the back of the Analyzer) until a reading of 0 C is obtained on the meter.	
8. Repeat the procedure with a water bath at 50 C except adjust meter with the Temp Adj screw ONLY.	8. Temp Adj screw is located on the left side (Figure 4) of the instrument case.
9. While pushing on the Temp Test button, turn the potentiometer screw that is second from the right (as you face the back of the Analyzer) until the meter reads 50 C.	

10. Using a 25 C water bath, compare the meter temperature reading with that of an accurate thermometer. If there is an error, readjust the potentiometer adjustment screw that is farthest to the right (as you face the back of the Analyzer).	10. The third potentiometer adjustment screw, which is located right of the multiplier adjustment screw, should never be turned.

Probe

Various DO saturation levels.

Before using the Analyzer, the analyst should calibrate the probe with samples whose DO concentrations have been determined by the Alsterberg (Azide) Modification of the Winkler Method. Calibrate the probe at least once every 3 weeks, preferably each day the Analyzer is used.

The DO probe is a partial pressure device; this means that the transfer of DO through the semipermeable membrane is a function of the ratio of DO concentration to DO concentration at saturation. For example, when a salt solution is saturated with oxygen at 20 C, it may contain only 3 ppm of DO; water, however, when saturated with oxygen at 20 C will contain 9.2 ppm of DO. Since the rate of oxygen transfer through the membrane would be the same in both cases, the probe output would be the same. The probe must therefore be calibrated using a liquid similar to the sample to be analyzed. Since quench water is greatly diluted with dilution water before analysis, the calibration of the probe should be performed with dilution water before analyzing general quench water samples. The probe must be calibrated each day it is used. If the membrane has been replaced, the calibration of the probe will change slowly for a period of about 24 hr before it stabilizes. If the probe is used during this period, frequent calibration checks will be required.

The probe must be calibrated to the specific turbulence of the system. Since the agitation is built into the probe assembly of the Weston & Stack BOD Agitator, any calibration that is performed is applicable to any container or use to which the BOD Agitator may be applied. (Note: The Modified Winkler Method must be established before the probe can be calibrated.)

Procedure	Comments
1. Saturate a liter of dilution water with oxygen.	1. This may be done by pouring the water back and forth from a graduate to a beaker at least three times.
2. Siphon the aerated water from the beaker into each of 3 BOD bottles.	2. During the siphoning, the water should be continuously stirred using a magnetic mixer and a Teflon-coated magnetic bar.
3. Using the Analyzer and probe, determine the DO and temperature of one of these aerated samples.	3. See Determination of the DO Concentration.
4. Adjust the calibration screw so that the Analyzer reads the same ppm DO as observed in Table 1 for the sample temperature.	4. a) See Figure 3 for the location of Cal screw. b) An exact DO concentration at 20 C and zero chloride concentration is 9.1 percent.
5. Determine the DO of the same sample using the modified Winkler Method.	5. See Reference 5.
6. Using the Analyzer and probe, determine the DO of the second aerated sample.	

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TABLE 1
SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR*†

Temperature (C)	Chloride concentration in water (mg/1)					Difference per 100 mg chloride
	0	5,000	10,000	15,000	20,000	
	Dissolved oxygen (mg/1–ppm)					
0	14.6	13.8	13.0	12.1	11.3	0.017
1	14.2	13.4	12.6	11.8	11.0	0.016
2	13.8	13.1	12.3	11.5	10.8	0.015
3	13.5	12.7	12.0	11.2	10.5	0.015
4	13.1	12.4	11.7	11.0	10.3	0.014
5	12.8	12.1	11.4	10.7	10.0	0.014
6	12.5	11.8	11.1	10.5	9.8	0.014
7	12.2	11.5	10.9	10.2	9.6	0.013
8	11.9	11.2	10.6	10.0	9.4	0.013
9	11.6	11.0	10.4	9.8	9.2	0.012
10	11.3	10.7	10.1	9.6	9.0	0.012
11	11.1	10.5	9.9	9.4	8.8	0.011
12	10.8	10.3	9.7	9.2	8.6	0.011
13	10.6	10.1	9.5	9.0	8.5	0.011
14	10.4	9.9	9.3	8.8	8.3	0.010
15	10.2	9.7	9.1	8.6	8.1	0.010
16	10.0	9.5	9.0	8.5	8.0	0.010
17	9.7	9.3	8.8	8.3	7.8	0.010
18	9.5	9.1	8.6	8.2	7.7	0.009
19	9.4	8.9	8.5	8.0	7.6	0.009
20	9.2	8.7	8.3	7.9	7.4	0.009
21	9.0	8.6	8.1	7.7	7.3	0.009
22	8.8	8.4	8.0	7.6	7.1	0.008
23	8.7	8.3	7.9	7.4	7.0	0.008
24	8.5	8.1	7.7	7.3	6.9	0.008
25	8.4	8.0	7.6	7.2	6.7	0.008
26	8.2	7.8	7.4	7.0	6.6	0.008
27	8.1	7.7	7.3	6.9	6.5	0.008
28	7.9	7.5	7.1	6.8	6.4	0.008
29	7.8	7.4	7.0	6.6	6.3	0.008
30	7.6	7.3	6.9	6.5	6.1	0.008
31	7.5					
32	7.4					
33	7.3					
34	7.2					
35	7.1					
36	7.0					
37	6.9					
38	6.8					
39	6.7					
40	6.6					
41	6.5					
42	6.4					
43	6.3					
44	6.2					
45	6.1					
46	6.0					
47	5.9					
48	5.8					
49	5.7					
50	5.6					

*Source: *Standard Methods for the Examination of Wastes and Wastewater.* 12th ed. Published, 1965 p. 409. Reproduced by permission, American Public Health Association, Inc., American Water Works Association, and Water Pollution Control Federation.

†At a total pressure of 760 mm Hg. Under any other barometric pressure, P (mm; or P', in.), the solubility, S' (mg/1), can be obtained from the corresponding value in the table by the equation:

$$S' = S\frac{P-p}{760-p}$$

in which S is the solubility at 760 mm (29.92 in.) and p is the pressure (mm) of saturated water vapor at the temperature of the water. For elevations less than 3,000 ft and temperatures below 25 C, p can be ignored. The equation then becomes:

$$S' = S\frac{P}{760} = S\frac{P'}{29.92}$$

Dry air is assumed to contain 20.90 percent oxygen. (Calculations made by Whipple and Whipple).

7. Considering the difference between the Analyzer and the Modified Winkler Method with the first aerated sample, adjust the calibration screw.	
8. Determine the DO of the same sample using the Modified Winkler Method.	
9. The third aerated sample is used as a re-check of the calibration point. If the calibration screw needs more adjustment, then more aerated sample should be prepared and analyzed until the calibration screw no longer needs adjustment.	9. After calibration, the probe should always be kept in a BOD bottle filled with distilled water to prevent air bubbles from entering the probe.

Having performed the above calibration procedure, the probe has now been calibrated at the upper and lower (zero adjustment of amplifier) DO saturation limits. The Analyzer's DO measurements are linear between these limits; however, the analyst may wish to verify the DO readings between these limits. This may be done by performing the probe calibration as just described except that pure nitrogen should be allowed to bubble for about 15 to 30 min through the aerated samples before the DO analysis. This treatment with nitrogen gas will lower the saturated oxygen concentration to about 3 to 5 ppm. The nitrogen gas will not interfere with the Modified Winkler Method's DO analysis.

System of known DO depletion capability.

The calibration procedures described thus far are usually employed only in the laboratory. The following method is applicable both in the laboratory and in the field where regular calibration procedures would be difficult to perform. This method uses a system of known oxygen depletion capability to evaluate indirectly the probe's response below the saturation level. It is not a true probe calibration method. The probe or Analyzer must first be calibrated by comparing its values with those obtained by the Modified Winkler Method; then, while it is being operated in the field, it can easily be checked for performance by the following method.

Procedure	Comments
1. Prepare 3 BOD bottles of aerated water samples as previously described in the probe calibration procedures.	
2. Using the analyzer and probe, determine the DO of one of these aerated samples.	2. See Determination of the DO Concentration.
3. Remove the probe and rinse it with distilled water.	3. When the probe is not being used, keep it in a BOD bottle that is filled with distilled water.
4. Using a volumetric pipet, add 2 ml of 0.25 M manganous sulfate solution to the same BOD bottle.	4. The tip of the pipet should be placed beneath the surface of the sample.

5. Immediately initiate the addition of 2 ml of 0.5 M potassium hydroxide solution; record the exact time of initiation.	5. a) Use a volumetric pipet. b) Use a stopwatch or other accurate device to measure exactly the 10-min reaction period.
6. After the KOH has been added, stopper the bottle and mix the contents by repeated inversions.	
7. After not more than 8 min have elapsed, insert the probe into the BOD bottle.	7. Equilibrium is attained in about 2 min.
8. After exactly 10 min have elapsed since the addition of the KOH solution, record the DO concentration.	
9. Repeat steps 2 through 8 with the other two prepared BOD bottles.	
10. Determine the depletion of DO for each sample by subtracting the final ppm DO from the initial observation.	10. The average depletion should be 3.60 ± 0.25 ppm. If the depletion is not this value, recheck calibrations; the instrument may have to be returned to the manufacturer.

ANALYSIS OF SAMPLES

Sample Collection

Site of collection.

When the BOD of quench water is measured to determine the amount of oxidizable wastes that will be discharged to a sewerage system serving an incinerator facility or to a drainage system associated with the residue disposal area, the site of sample collection must be chosen with due consideration. Settlement tanks, surface pools, sewers, and other areas immediately adjacent to the sewage or drainage system are preferable collection sites.

Sample size and container.

Normally, 50 ml of sample is needed to perform the BOD analysis; however, since various dilutions may be needed, and a larger sample size may be more representative, it is recommended to collect 1 liter of quench water sample.

The samples should be collected in sterile, unbreakable bottles with narrow mouths and caps that can be tightly fitted. The sample bottle should be completely filled. All containers must be thoroughly rinsed, especially if cleaned with a detergent, before they can be reused.

Samples should not be collected on Monday or Tuesday unless the analysts are to work on Saturday or Sunday (5-day BOD).

Sample Preservation and Shipment

If the sample analysis is to be initiated within 4 hr after collection, sample preservation measures are not absolutely necessary. Samples should otherwise be placed in an ice chest (or similar container)

soon after collection so that the samples are maintained in the dark at 5 C. The bottle caps must be tightly fitted to prevent an increase in oxygen solubility with the reduction in temperature.

Sample shipment to the laboratory should be immediate (via air freight if necessary) to ensure the initiation of BOD analysis in the laboratory within 24 hr of sample collection. Samples that are more than 24 hr old should not be analyzed.

Since the Analyzer requires calibration, the laboratory personnel should be informed at least 2 days before the arrival of samples. Because samples require some preparation before the actual analysis and since the exact dilution requirements may not be known, samples not at 5 C should be shipped to the laboratory so that they are received at least 2 hr before the end of the normal working day. Samples shipped in an ice chest at 5 C and then refrigerated may be analyzed the following day.

Sample and Blank Preparation

Adjustment for nitrification process.

Before analysis, each sample and dilution water blank is treated as follows to inhibit the nitrification process:

Procedure	**Comments**
1. Place 50 ml of a thoroughly mixed quench water sample in a 250-ml beaker.	**1. The exact volume of quench water sample depends upon the dilution requirements. See Dilution and Aeration.**
2. Using pH paper, check the pH of the sample.	**2. Usually the pH is about 11.**
3. Using 1 N NaOH or 1 N H_2SO_4, adjust the pH of the sample to a range of 2 to 3; maintain pH for 15 min.	**3. Omit if the sample already has a pH of 2 or 3.**
4. Then neutralize the sample to a pH of 6.5 to 8.3.	**4. Employ the same 1 N solutions as in step 3.**

Adjustment for residual chlorine.

Chlorine, in concentrations normally found in chlorinated water and sewage effluent, does not influence the probe output or the determination of oxygen. Chlorine will react with lead and hence cause the probe sensitivity to decrease after long exposure. Since residual chlorine dissipates when samples stand for 1 to 2 hr or are well aerated, no adjustments are recommended.

Dilution and aeration.

Prepared samples must be diluted in order to obtain a measurable depletion (2 ppm to 7 ppm) of oxygen at the end of the 5-day incubation period. Since incinerator quench water usually has a BOD of 100 to 300 ppm, a suitable or applicable dilution is 50 ml of sample diluted to 2 liters. If the analyst suspects that the BOD of the quench water differs from the usual value, he should test various dilutions since the analysis cannot be repeated on the same original sample after the 5-day waiting period. To obtain more reliable results, 3 BOD bottles should be prepared: 3 for the initial DO, and the same 3 for the final DO. The final DO values should never be less than 1.0 ppm.

Since the dilution water used in the analysis of each quench water sample may contain a few oxidizable materials capable of exerting a small BOD, each quench water analysis should include a

blank evaluation, i.e., a determination of the BOD of the dilution water. The observed BOD of the quench water can then be corrected by subtracting the appropriate proportionate fraction of this blank value.

The dilution and aeration procedures are as follows:

Procedure	Comments
1. Pour the total prepared sample from the 250-ml beaker into a 2-liter graduate and dilute to the mark with dilution water.	1. Solution still represents 50 ml of original sample.
2. Aerate the sample by pouring it back and forth from the graduate into a 3-liter beaker at least 3 times.	2. The dilution water blank is aerated in a like manner.
3. Siphon the diluted, aerated sample or blank from the beaker and into 3 BOD bottles.	3. The sample should be stirred continuously, using a magnetic stirrer and a Teflon-coated magnetic bar.
4. The DO concentration of the sample or blank in the 3 BOD bottles should be determined immediately.	4. a) See Determination of the DO Concentration. b) Only two reasonable DO results are needed. See Precision.
5. Then put the same 3 BOD bottles in an incubator (or waterbath) and determine their DO content after a 5-day incubation period at 20 C.	5. a) During the incubation period, the samples should not be exposed to the light. b) See Determination of the DO Concentration. c) Only two reasonable DO results are needed. See Precision.

Determination of the DO Concentration

The Analyzer has the components necessary for operation when shipped. Before attempting to place the analyzer into service, check to ascertain that a) the membrane is free of holes, b) the needle zeroes correctly, c) the temperature reading is correct, and d) the probe is free of air bubbles.

Procedure	Comments
1. Turn the toggle switch located in the lower left-hand corner of the front panel to "<5."	1. In most cases the entire body of the probe is essentially at the temperature of the sample. If the probe body temperature varies more than 5 C from the sample temperature, turn the toggle switch to "<5."
2. Turn the selector switch to DO-Mult 1.	
3. Turn toggle switch located in front lower right hand corner of case to ON.	

4. Insert probe into BOD bottle.	**4. The agitator should be operating.**
5. Wait 2 min for equilibrium and then read the ppm dissolved oxygen directly on top of scale.	**5. a) Record value.** **b) The absolute value of the difference between duplicated readings should not exceed $1.96\sqrt{2}$ s, or 0.58 ppm, more than 5 percent of the time. See Precision.**
6. If temperature of the sample is desired, turn selector switch to Temp and read temperature (C) directly on the bottom scale.	**6. a) Record value.** **b) Accuracy is ± 1 C.**
7. Turn toggle switch (right-hand corner) to OFF and selector switch to TRANSIT.	**7. Perform this step if the meter is not to be used for several hours.**

CALCULATIONS

BOD of Dilution Water

The following formula should be employed to calculate the BOD of each individual sample of dilution water.

$$BOD_1 = D_1 - D_2$$

where

BOD_1 **= The BOD of dilution water**
D_1 **= The DO content of initial (before incubation) dilution water**
D_2 **= The DO content of final (after incubation) dilution water**

BOD of Quench Water

The initial DO concentration minus the final DO concentration equals BOD of the diluted sample. The BOD of the diluted sample times the dilution factor equals the BOD of the original sample.

The dilution factor is found by dividing the original amount of sample taken into the final dilution; for example, 50 ml of sample diluted into 2 liters gives a factor of 40.

The following formula should be used to calculate the BOD of each individual sample of quench water.

$$BOD_2 = F[(D_3 - D_4) - P_1(BOD_1)]$$

where

BOD_2 **= The BOD of quench water**
F **= The dilution factor**
D_3 **= The DO content of initial (before incubation) quench water**
D_4 **= The DO content of final (after incubation) quench water**
P_1 **= The decimal fraction of dilution water used in the BOD analysis of the quench water**

METHOD EVALUATION

Precision

After analyzing a number of quench water samples in duplicate (three determinations were performed to ensure reasonable duplicate results), the precision of the observations were evaluated by calculating (using the Olivetti Programma 101) the pooled standard deviation of all observations except those obtained on samples collected from dump truck drainage. The results of these calculations are shown as follows:

Precision of the DO Analysis:*	
Number of determinations†	82
Pooled standard deviation (s) ‡	0.21
Confidence interval ± $1.96\sqrt{2}$ (s) §	±0.58

Precision of the BOD Analysis:	
Number of determinations	20
Standard deviation (s) ¶	0.30
Dilution factor #	40
Confidence interval ± 1.96 (40) S**	±23.5

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*Assistance in the statistical analysis was provided by the Statistical Section of the Office of Solid Waste Management Programs.

†Normally, at least two initial and three final determinations were made for each sample.

‡A pooled standard deviation was computed for all determinations. It was assumed that there was no statistically significant difference between initial and final variances; i.e., homogeneity of the variances was assumed.

§The absolute value of the difference between duplicated readings should not exceed $1.96\sqrt{2}$ (s), or 0.58 ppm, more than 5 percent of the time. The covariance between the duplicated readings was ignored.

¶The formula for the standard deviation of the difference between initial and final DO readings is $S = \sqrt{(s^2 + s^2)}$. In this calculation it was assumed that the initial and final pooled variances were equal, and the covariance term between initial and final readings was ignored.

#The dilution factor may vary, but for calculation purposes, the normal dilution factor is shown here.

**The confidence limits for a single BOD result are 95 percent, assuming a standard dilution factor of 40, or 2.5 percent dilution.

Accuracy

There is no standard with which the accuracy of the determination can be measured. The accuracy of the instrument is 1 percent of the reading and is better than ± 0.1 ppm.

Sensitivity

This DO Analyzer method is not applicable to samples with a dilution factor of 40 that have a 5-day BOD value of 23.5 ppm or less.

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METHODS FOR DETERMINING CELLULOSE IN COMPOST*

Richard D. Lossin†

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*Reprinted from COMPOST SCIENCE Journal of Waste Recycling, 12 (1) : 12-13, January-February 1971.

†At the time this study was performed, Mr. Lossin was a Research Chemist with the now Solid Waste Research Laboratory, National Environmental Research Center, Cincinnati.

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INTRODUCTION

The major constituent of municipal refuse is cellulose, which is degraded by microorganisms in the composting process to yield a humus-like, stable product usable as a soil conditioner or mulch. It was necessary, therefore, to develop methods for the determination of cellulose as part of the in-house research on the characterization of compost and the composting process at the U. S. Public Health Service–Tennessee Valley Authority Composting Project at Johnson City, Tennessee. The two methods presented here are modifications of two methods previously reported (1, 2) and were found to be accurate and reproducible for all analyses performed on compost.

ANTHRONE COLORIMETRIC METHOD

Reagents

1. Diluted H_2SO_4, reagent grade
   (760 ml concentrated H_2SO_4 + 300 ml water)
2. Anthrone reagent: 1 g anthrone in 500 ml cold, 96-percent H_2SO_4;
   let stand at room temperature 4 hr before use
3. Benzene, reagent grade
4. Pure cellulose standard (Whatman No. 1)

Procedure

Weigh out a finely ground (2-mm mesh) and redried compost sample to the nearest milligram, place in a Soxhlet extractor, and extract with benzene for 8 hr. Dry the sample and weigh it in order to compute the percent material extracted with benzene. Extract this sample again with hot water for 8 hr. Dry the sample and weigh it in order to compute the percent material extracted with water. Take between 0.5 to 1.0 g of the dried sample weighed to the nearest milligram and place it in a 250-ml beaker; wet it with a few drops of 95-percent ethanol, methanol, or acetone. Pipette in 10.0 ml water, then 60.0 ml diluted sulfuric acid, and stir to dissolve the cellulose. After about 5 min, pipette exactly 10.0 ml of the cellulose solution into a 500-ml volumetric flask and dilute to 500 ml. Pipette 1 ml of this into a test tube, add exactly 10.0 ml anthrone reagent, mix, cap the tube to prevent the steam from condensing on the inside of the tube, and heat in a 100 C bath for 15 min. Cool to room temperature and read the absorbance at 630 mμ. Run cellulose standards to bracket the sample concentration and a blank with each series of samples. A blank and standards must be included with each group of samples heated in the 100 C bath.

Calculations

$$\%\ \text{cellulose} = \frac{(\text{g cellulose found}) \times [100 - (\%\ \text{benzene extract} + \%\ \text{water extract})]}{\text{g of extracted sample}}$$

If all the sample can be recovered and used after the benzene and water extractions, it is unnecessary to compute the percent of extracted material. Simply know the initial weight of the sample, the number of grams found (from the anthrone standard curve), and compute the percent cellulose from this. This is easily accomplished by placing 0.5 to 1.0 g of compost in a porcelain thimble with an asbestos filter, capping with glass wool, extracting, and then washing all the sample into a beaker and proceeding with the determination as before.

GRAVIMETRIC METHOD

Reagents

All of the following should be reagent grade:

1. Concentrated nitric acid
2. Glacial acetic acid
3. Benzene
4. Ether
5. Methanol
6. Acetone

Procedure

Weigh out about 1 g of redried, finely ground compost to the nearest milligram, place in a 125-ml Erlenmeyer flask, and add 6 ml water, 24 ml glacial acetic acid, and 2 ml concentrated nitric acid. Bring to a gentle boil on a hotplate for 20 min, cool to about 80 C, add 50 ml benzene, and swirl vigorously for about 2 min to extract materials soluble in benzene. Set up a Gooch crucible with an asbestos filter on a suction flask; decant as much of the benzene layer as possible into the filter (with suction), taking care not to let the bottom layer spill over. Then add 50 ml of ether to the flask, swirl vigorously, let settle, and decant all the liquid into the crucible. Wash all the solid material into the crucible with acetone, taking care not to leave any behind. Wash the filter cake thoroughly with successive 100-ml portions of hot benzene, hot methanol, and ether. After washing, clean the outside of the crucible, place in an oven to dry, cool in a desiccator, weigh to the nearest milligram, and ignite at 625 C in a muffle furnace for 1 hr. Cool, weigh, and report the loss after ignition.

Calculations

$$\frac{\text{loss after ignition x 100}}{\text{initial sample weight}} = \text{percent cellulose}$$

DISCUSSION

Duplicate samples should always be run to ensure precision.

Swirling the benzene with the hot reaction mixture is necessary to ensure rapid filtering of the successive solvents used. Compost contains a tar-like material that plugs the filter, and most of this is soluble in benzene.

An alternate approach is provided by combining the two methods. Complete the solvent washings as in the gravimetric method, then dissolve the entire sample, as in the anthrone method, and determine the cellulose colorimetrically. This was done three times with a 0-day composite compost sample, and the results were 49.4, 49.4, and 49.6 percent, respectively. The gravimetric method on the same sample yielded 50.2, 50.0, and 50.3 percent, respectively. The higher results indicate either that (a) some substances were not removed by the extractions (0.7 percent) and were not cellulose, but were lost after ignition, or that (b) there was some interfering material in the sample that suppressed the anthrone reaction slightly.

Analyses were performed (Table 1) on samples to which known amounts of cellulose had been added to check the gravimetric method further. The graph in Figure 1 shows the standard addition results for the 0-day compost.

TABLE 1

ANALYSES OF SAMPLES CONTAINING KNOWN AMOUNTS OF CELLULOSE

Type of sample	Cellulose (%)				
0-day compost:					
Found amount	50.3	55.1	60.6	65.4	
Theoretical amount		55.2	60.5	65.4	
56-day compost:					
Found amount	24.3	37.6	29.4	60.9	
Theoretical amount		36.8	28.7	61.5	
1-year compost:					
Found amount	19.1	28.0	38.3	48.1	60.8
Theoretical amount		28.2	39.1	49.2	61.1

The gravimetric method is recommended because it is more rapid and easier to perform than the anthrone colorimetric method.

It is often difficult to obtain a representative sample from compost because of its nonhomogeneity. For an analysis to be valid, it is necessary to work with a representative sample; that is, the overall characteristics of the sample must be similar to the part being sampled. To this end, the following sampling procedure is outlined:

From the digestor or windrow, select small grab samples (50-100 g) randomly to make a total composite sample of at least 1 kg—the larger the sample, the better. This sample should be rough-ground (by W-W grinder, hammermill, etc.) and thoroughly mixed; duplicate samples are taken from this for testing. In general, the representative character and homogeneity of the sample are improved by the following: (1) increasing the sample size, (2) taking larger numbers of composites, (3) grinding finer, and (4) mixing better.

The reliability of the testing procedure can be checked by taking several different samples from the same sample population (compost pile) and performing the same test on all. If they are not all nearly the same, then the sampling technique should be modified to correct any gross fluctuations.

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MEASUREMENT OF THE CHEMICAL OXYGEN DEMAND OF COMPOST*

Richard D. Lossin†

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*Reprinted from COMPOST SCIENCE Journal of Waste Recycling, 12 (2) : 31-32, March-April 1971.

†At the time this study was performed, Mr. Lossin was a Research Chemist with the now Solid Waste Research Laboratory, National Environmental Research Center, Cincinnati.

INTRODUCTION

Microorganisms are able to utilize municipal refuse as a growth substrate, eventually degrading it to the humus-like substance we call compost. During this process the refuse is oxidized by the microorganisms to yield carbon dioxide, water, and heat as the primary products of their metabolism. We can measure the extent of biological oxidation in the system by measuring the extent to which the refuse (compost) is oxidized by chemical means. This ability to be oxidized is called the chemical oxygen demand or COD.

Currently there is no published standard method for the determination of the COD of compost. The procedure presented here was developed after extensive research at the U. S. Public Health Service–Tennessee Valley Authority Composting Project at Johnson City, Tennessee, as part of the in-house research on the characterization of compost and the composting process.

REAGENTS

1. 1.000 N potassium dichromate, reagent grade
2. Standardized ferrous ammonium sulfate, reagent grade
3. Concentrated sulfuric acid, reagent grade
4. Ferroin indicator (orthophenanthroline)

PROCEDURE

Weigh out 0.2 to 0.3 g of finely ground, dry compost (2-mm mesh) to the nearest milligram and place the sample in a 250-ml flask fitted with a reflux condenser. Pipette in exactly 50.00 ml 1.000 N $K_2Cr_2O_7$ and add 30 ml distilled water and 20 ml concentrated H_2SO_4. Reflux this mixture gently for 1 hr. (Equally good results were obtained with a 250-ml Erlenmeyer flask and a hotplate instead of a reflux apparatus, the water lost by evaporation being periodically replaced.) Cool the reflux mixture, dilute to 250 ml in a volumetric flask, mix well, pipette 10.00 ml into a 500-ml Erlenmeyer flask, and add about 100 ml water, and 20 ml of concentrated sulfuric acid. Titrate with standardized ferrous ammonium sulfate (about 0.1 to 0.2 N) using ferroin as an indicator. Standard Methods for the Examination of Water and Wastewater (12th ed., 1965, p. 510-514) should be consulted for techniques and standardization procedures.

CALCULATIONS

$$\text{COD} = \frac{(A - B) \times C \times 200}{\text{g of sample}} \quad \text{(in mg/g)}$$

where

A = ml of titrant used for blank

B = ml of titrant used for sample

C = normality of the titrant

The constant 200 is derived as follows:
Standard Methods defines COD as follows:

$$\text{COD} = \frac{(A - B) \times C \times 8{,}000}{\text{ml sample}} \quad \text{(in mg/liter)}$$

Here 10 ml of sample is used, and the total sample size is 250 ml, or ¼ liter. The expression now becomes:

$$\mathrm{COD} = \frac{(A - B) \times C \times 8{,}000 \times \frac{1}{4}}{10\ \mathrm{ml}} \quad \text{(in mg/250 ml)}$$

or

$$\mathrm{COD} = (A - B) \times C \times 200 \quad \text{(mg/250 ml)}$$

Since the entire sample has been diluted to 250 ml, we simply substitute the grams of sample used to obtain the answer in milligrams per gram.

DISCUSSION

It is advisable to run a duplicate of the original sample and a duplicate of each dilution. This investigator found very good precision in duplicate assays, and this indicates that the method is stable and reproducible for a given sample of compost. This observation is further substantiated by comparing the COD (in mg per g of randomly selected windrows) with the age of the compost (Figure 1). Johnson City compost is considered stable and ready for use at 8 weeks, which corresponds to a COD of less than 700 mg per g as opposed to about 900 mg per g for fresh refuse.

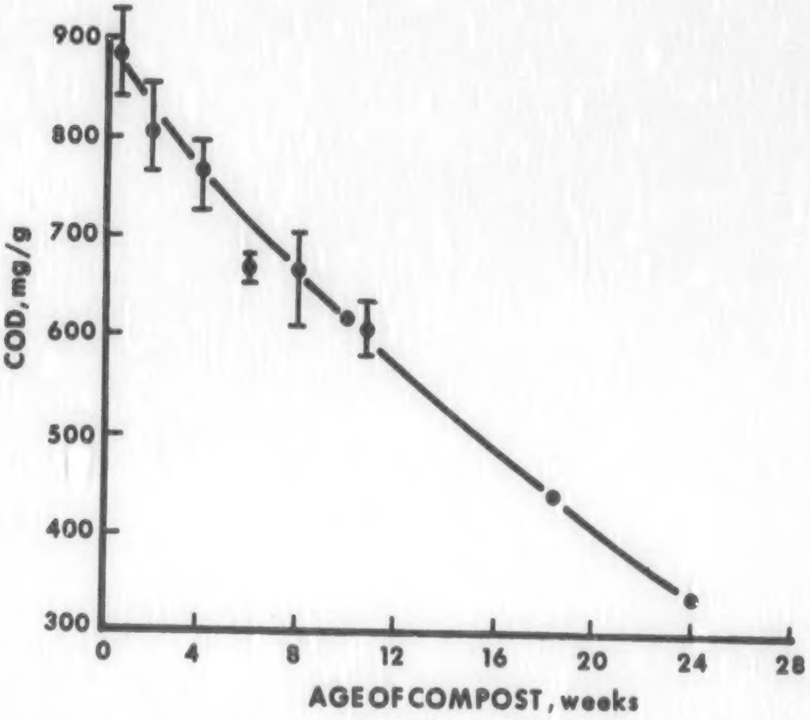

Figure 1. COD of randomly selected windrows versus age.

It is often difficult to obtain a representative sample from compost because of its nonhomogeneity. For an analysis to be valid, it is necessary to work with a representative sample; that is, the overall characteristics of the sample must be similar to the part being sampled. To this end, the following sampling procedure is outlined:

From the digestor or windrow, select small grab samples (50-100 g) randomly to make a total composite sample of at least 1 kg; the larger the sample, the better. This sample should be rough ground (by W-W grinder, hammermill, etc.) and thoroughly mixed; duplicate samples are taken from this for testing. In general, the representative character and homogeneity of the sample are improved by the following: (1) increasing the sample size, (2) taking a larger number of composites, (3) grinding finer, (4) mixing better.

The reliability of the testing procedure can be checked by taking several different samples from the same sample population (compost pile) and performing the same test on all of them. If they are not all nearly the same, then the sampling technique should be modified to correct any gross fluctuations.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Donald J. Dunsmore for his assistance in the initial work in developing this procedure.

QUALITATIVE DETERMINATION FOR THE DEGREE OF DECOMPOSITION OF COMPOST BY THE STARCH-IODINE METHOD*

Richard D. Lossin†

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*Reprinted from COMPOST SCIENCE Journal of Waste Recycling, 11 (6) : 16-17, November 1970.

†At the time this study was performed, Mr. Lossin was a Research Chemist with the now Solid Waste Research Laboratory, National Environmental Research Center, Cincinnati.

INTRODUCTION

A simple and rapid test has long been needed for determining the degree of decomposition of compost. Many tests have been proposed and used, including measurement of the reduction in total carbon or in the carbon-nitrogen ratio, increase in percent ash, decrease in cellulose, and decrease in lipids. These methods are adequate for determining the completeness of composting on the assumption that a representative sample has been taken. All have, however, obvious disadvantages: each requires complicated sample preparation, considerable time, and, in most cases, expensive equipment.

This investigator has always found starch in measurable quantities (2 to 6 percent) in municipal refuse. Since starch is in the class of easily degraded substrates that must be broken down and metabolized before the refuse becomes a microbiologically stable product, testing for its presence will indicate whether or not the compost is stabilized. The rationale of the test rests on the hypothesis that all refuse contains a measurable quantity of starch and that the starch must be degraded before the compost can be considered acceptable, no matter how much was initially present. The test discussed here is based on the formation of the starch-iodine complex in an acidic extract of compost and has the following advantages: It is rapid and easily performed, it is specific for starch, and the equipment needed is very simple.

Although the method has not been tested on different composts, it was valid for compost from the Joint Public Health Service–Tennessee Valley Authority Composting Project in Johnson City, Tennessee. The method is presented here to aid other workers in the field.

THEORY

Three types of carbohydrates are found in compost: sugars, starch, and cellulose (Figure 1). These constituents decrease with age of the compost (Figures 2-4). Sugars are metabolized first, being almost completely consumed within a week; starches are next, followed by cellulose. By the fourth to fifth week of windrow composting, the starch has passed through its maximum rate of degradation. This is the stage in the process where the compost first has an acceptable appearance and odor, has gone through its maximum temperature phase, and has reached its maximum pH (Figures 5 and 6). In other composting processes with different types of refuse, these events may occur at different time intervals, but the relationships can be expected to be approximately the same.

Certain polysaccharides (starches: amylose, amylopectin, glycogen, and dextrans) form characteristic color complexes when combined with molecular iodine (1-3). Amylose (straight-chain starch) gives an intense blue-black color; amylopectin (moderately branched) gives from light blue to purple to red colors, depending on the degree of branching; and glycogen (highly branched) gives reddish-brown colors. Dextrans, which are partial degradation products of starch, give reddish-brown to yellow colors. The hydrolysis of starch may be followed by a gradual change in the iodine complex color (blue-black to light blue to purple to red to yellow to no color).

In the present study, a test was run to determine whether all of the starch found in the compost was complexing with iodine. The weight of starch-iodine complex recovered from 10-g compost samples varied with different compost ages. This corresponded very well with determinations of the amount of starch in the compost.

PROCEDURE

The iodine reagent is prepared by dissolving 2.00 g KI in 500 ml H_2O, then dissolving 0.80 g I_2. The other reagent is perchloric acid (36 percent).

Place about 1 g compost (see Discussion) in a 100-ml beaker, wet with a few drops of ethanol if dry, add 20 ml perchloric acid (36 percent), and stir. Filter through open-texture filter paper (Whatman No. 90). Add 2 ml iodine reagent to the filtrate and stir. Place a few drops on a spot plate or

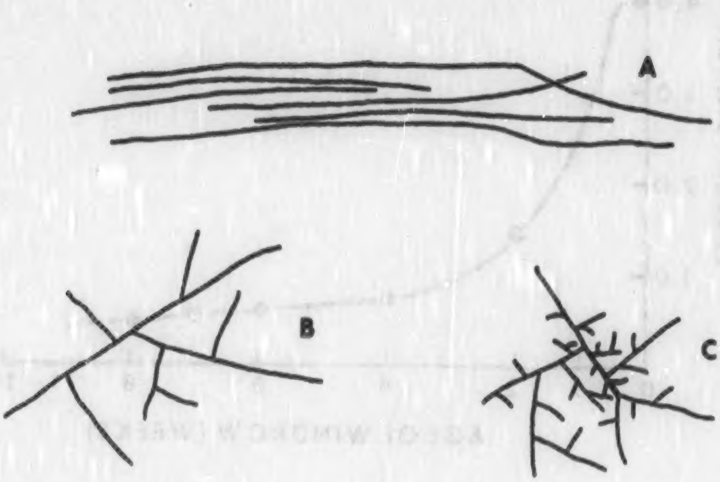

Figure 1. Amylose (A), amylopectin (B), and glycogen (C).

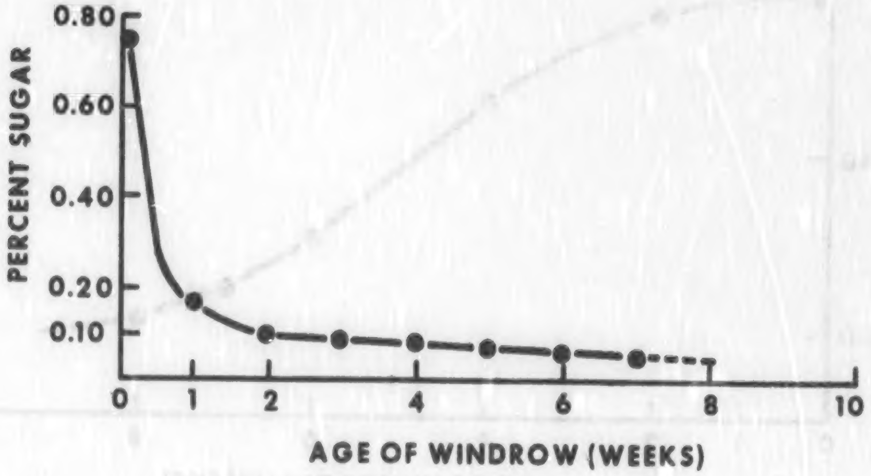

Figure 2. Decrease of sugar versus age of compost.

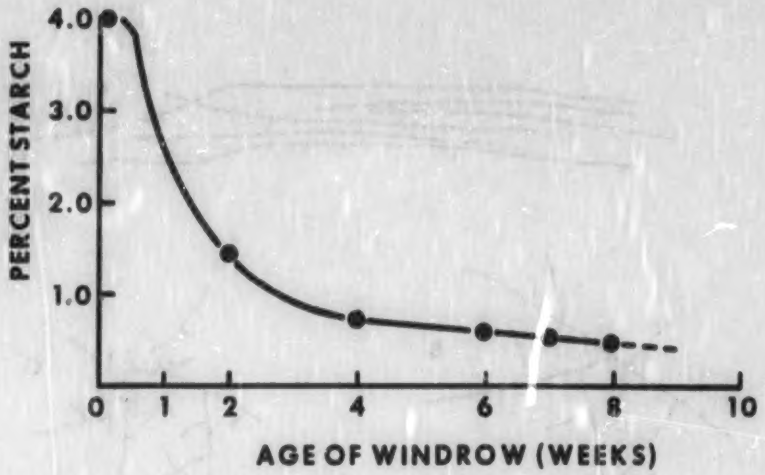

Figure 3. Decrease of starch versus age of compost.

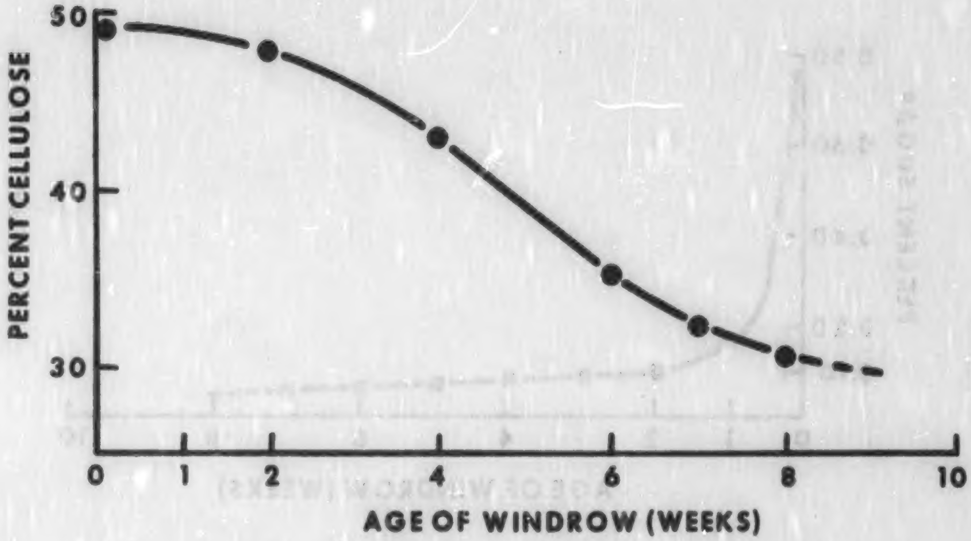

Figure 4. Decrease of cellulose versus age of compost.

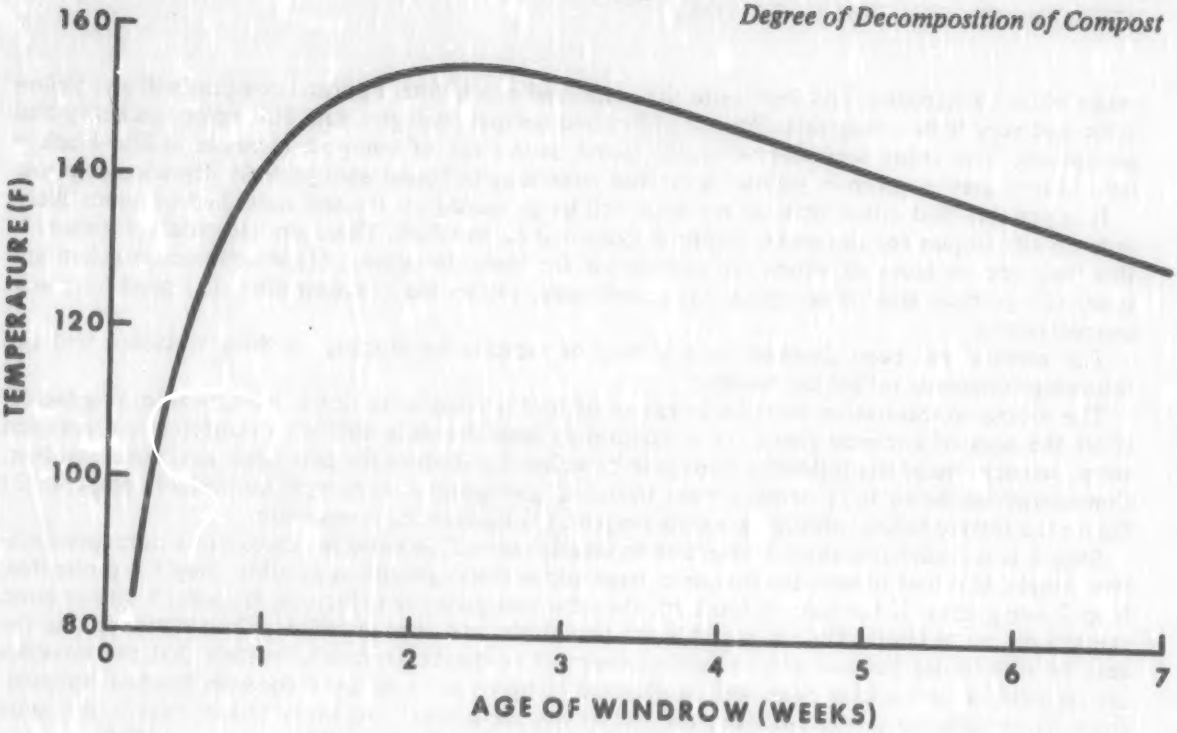

Figure 5. Temperature versus age of compost.

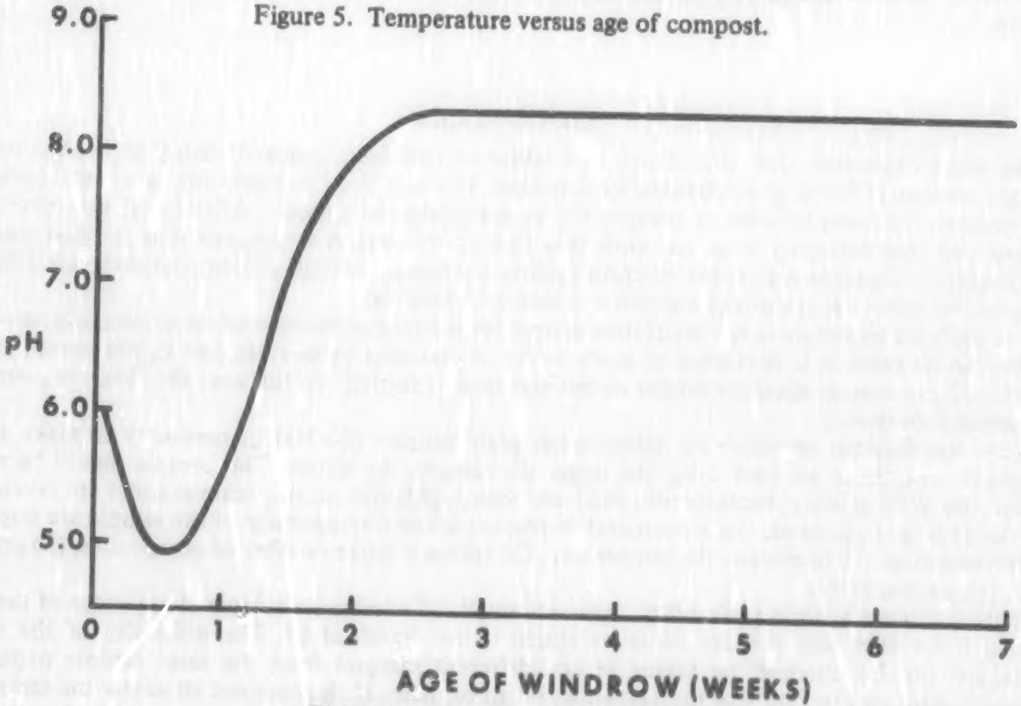

Figure 6. pH versus age of compost.

other white background, and then note the color and precipitate. Finished compost will give yellow color and very little precipitate. Poor or unfinished compost will give dark blue color and heavy blue precipitate. The color sequence normally found as the age of compost increases is blue-black → light blue → grey → green → yellow. A reddish color may be found with partially degraded compost.

It is possible that either little or no color will be produced by the test described or, more likely, tests on all samples for the entire compost cycle will be too dark. There are five variables present in this test, one or more of which are responsible for these deviations: (1) starch concentration and type, (2) particle size of compost, (3) sample size, (4) iodine concentration, (5) perchloric acid concentration.

The method has been checked on a variety of samples by altering all these variables, and the following comments reflect the results.

The iodine concentration must be in excess of that needed so as not to become a limiting factor. If all the ages of compost give a color reaction so dark that it is difficult to differentiate between them, one or more of the following steps may be taken: (1) Reduce the perchloric acid concentration. Concentrations down to 5 percent were found to give positive tests with unfinished compost. (2) Dilute the filtrate before adding the iodine reagent. (3) Reduce the sample size.

Step 3 is not advisable since a reduction in sample size will increase the chance of a nonrepresentative sample. It is best to have the sample as large and as finely ground as possible. Step 1 is preferable, Step 2 being next. If the color is too light, then the best policy is to increase the sample size or grind the sample more finely. Do not increase the perchloric acid concentration. When standardizing the test, be sure to use finished and unfinished compost as standards, making certain that the variables are adjusted to give a blue color with unfinished compost and a yellow color with finished compost. *Once these variables are established, they must not be altered.* Do every test in exactly the same fashion.

DISCUSSION

One must remember that this is only a qualitative spot test, designed simply to show a relative change; nothing is being quantitatively determined. The test is to be used only as a control measure for checking the completeness of composting in one particular process. After one has checked satisfactory and unsatisfactory compost with this test, it will become apparent that finished compost always gives a characteristic color reaction (yellow), whereas unfinished compost does not. Finished compost has never given a positive starch reaction with this test.

It is difficult to obtain a representative sample from compost because of its nonhomogeneity. For the test to be valid, it is necessary to work with a representative sample; that is, the overall characteristics of the sample must be similar to the part being sampled. To this end, the following sampling procedure is outlined:

From the digestor or windrow, select small grab samples (50-100 g) randomly to make a total composite sample of at least 1 kg; the larger the sample, the better. This sample should be rough-ground (by W-W grinder, hammermill, etc.) and thoroughly mixed; duplicate samples are taken from this for testing. In general, the representative character and homogeneity of the sample are improved by the following: (1) increasing the sample size, (2) taking a larger number of composites, (3) grinding finer, (4) mixing better.

There is always a finite probability, however small, of selecting a sample at any stage of the composting cycle that will contain excessive starch or no starch at all. The reliability of the testing procedure can be checked by taking several different samples from the same sample population (compost pile) and performing the same test on all of them. If they are not all nearly the same, then the sampling technique should be modified to correct any gross fluctuations.

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VACUUM-ACID HYDROLYSIS OF FUNGAL PROTEIN AND PROTEIN FROM OTHER SOURCES

W. Emile Coleman*

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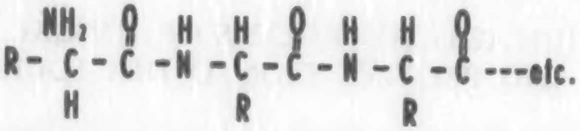
DISCUSSION

All proteins yield amino acids when hydrolyzed, since a protein molecule may contain hundreds or thousands of amino acid groups. The amino acids are united through an acid amide type of bond called a peptide linkage $(-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-)$. The nature of the peptide linkage between the amino acids of the protein is shown below.



$$R-\underset{H}{\overset{NH_2}{C}}-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-\underset{R}{\overset{H}{C}}-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-\underset{R}{\overset{H}{C}}-\overset{O}{\overset{\|}{C}}\text{---etc.}$$

Just as is the case with acid amides, the peptide linkages in proteins are resistant to hydrolysis and require prolonged boiling with relatively strong acids or alkalies for complete liberation of each amino acid. The acids most commonly used are hydrochloric (6 N), sulfuric (8 N), and hydriotic (57 percent). Among bases, 5 N NaOH and 14 percent $Ba(OH)_2$ are commonly used. Acid hydrolysis of proteins is generally preferred. Alkaline hydrolysis, although efficient in liberating the amino acids, partly converts the pure, optically active forms that exist in proteins into racemic mixtures (mixtures of D and L forms) (1). Acid hydrolysis is essentially free from this objection. Both acid and alkaline hydrolysis lead to some decomposition and loss of the more unstable amino acids.

The developed technology for producing fungal protein in the Solid Waste Research Laboratory is the result of research efforts directed toward recycling both starchy and cellulosic wastes (2, 3). The quality of the protein produced by fermentation depends on the amino acid profile that was determined by a quantitative amino acid analysis with an Automatic Amino Acid Analyzer (4). Before an analysis, however, hydrolysis of the protein must take place to produce individual amino acids.

The subject procedure uses acid hydrolysis under vacuum conditions. The use of a vacuum eliminates the oxidizing atmosphere, which would result in oxidation or loss of the amino acids. This hydrolysis procedure can be applied to any protein-bearing material, plant, or animal.

APPARATUS

1. Vacuum pump, capable of obtaining 50-100μ of pressure
2. Pressure gauge, range of 50μ to 1 atm.
3. Cold trap
4. Dewar flask containing dry ice with acetone
5. Evaporating dishes (one per sample)
6. Glass funnels (one per sample)
7. Filter paper, #40
8. Constant temperature oven, thermostatically controlled at 110C ± 1C
9. All-glass pressure system as shown in Figure 1
10. Analytical balance
11. Pipet, 10-ml, one
12. Vacuum hydrolysis tubes (one per sample) (4)

13. Hi-Vacuum grease
14. Filtering flasks, 50-ml, one per sample

REAGENTS

1. Hydrochloric acid, reagent grade
2. Prepurified nitrogen, one cylinder

Preparation of Solutions

Hydrochloric acid, 6 N: Add equal volumes of distilled water and concentrated HCl. One liter of solution is sufficient.

SAFETY PRECAUTIONS

When presented to the analyst for protein analysis, the fungal growths are usually still active or "alive." Before attempting analysis, the molds (fungal growths) should be autoclaved for 15 to 20 min at 120 C.

SAMPLE PREPARATION

The fungal protein is usually grown in a 500-ml flask containing 100 ml of growth media. Before analysis, the fungal mass is treated as follows:

Procedure	Comments
1. After the fungal mass has been autoclaved, decant the growth media and retain the mold in the flask.	1. The growth media can be discarded.
2. Add approximately 200 ml of distilled water to the flask.	2, 3, 4. This washing procedure is vital because it ensures removal of all salts remaining from the growth media. If not removed, the metal ions in the growth media become bonded to the cation exchange resin in the chromatographic column and thus interfere with the analysis. Such a condition adversely affects the capacity of the resin to attract amino acid molecules, thus resulting in loss of resolution. In addition, if the ammonium ion (an ingredient of the media) is not removed by washing, it produces a huge peak that covers up several of the amino acids on the chromatogram.
3. Wash the mold in distilled water by shaking for about 5 min, then decant the liquid.	
4. Repeat steps 2 and 3 twice.	
5. After the last washings are decanted, pour the mold onto a funnel containing coarse filter paper.	5. Whatman 40 filter paper will suffice.

6. Transfer the mold from the filter paper into an evaporating dish.	6. Shaking the paper will dislodge the mold.
7. Place evaporating dish in an oven thermostatically controlled at 75 C for 24 hr or freeze dry the material.	7. The analyst should plan his time so that this step can be accomplished by 9:00 am.
8. At the end of 24 hr obtain and record the dry weight of the fungal protein.	
9. With mortar and pestle, grind the dried mold until uniform in size.	

DEAERATION AND HYDROLYSIS

Procedure	Comments
1. Weigh out 10 - 20 mg of the dried protein material into the bottom section of a reusable vacuum hydrolysis tube.	1. The two-section hydrolysis tube can be seen in Figure 1.
2. Pipet 10 ml of the 6 N HCl into the hydrolysis tube.	
3. Lightly coat the ground-glass ball joint and stopcock of the hydrolysis tube with high vacuum grease; then clamp securely.	3. Use Dow – Corning or Apiezon H.
4. Close stopcock on top section of vacuum hydrolysis tube and connect to pressure system as shown in Figure 1.	
5. Start the vacuum pump and evacuate pressure system below 50μ of Hg pressure.	
6. SLOWLY open the stopcock on the hydrolysis tube to deaerate the mixture. Observe when pressure gauge reaches 50 - 100μ of Hg pressure.	6. If the stopcock is opened too suddenly, the sample will be pulled into the vacuum lines. A slight tap on the side of the tube will help to dislodge air bubbles when the vacuum is applied.
7. Close the stopcock on the hydrolysis tube and shut off the vacuum from the pressure system.	7. A large stopcock is installed in the system to isolate the vacuum pump.
8. Flush the system with nitrogen for about 3 min.	8. Nitrogen pressure at the point of entry to the system is 2-3 PSI.
9. Open the stopcock on the hydrolysis tube and allow tube to fill with nitrogen.	9. Two min is sufficient.
10. Close stopcock on vacuum hydrolysis tube and shut off nitrogen supply.	

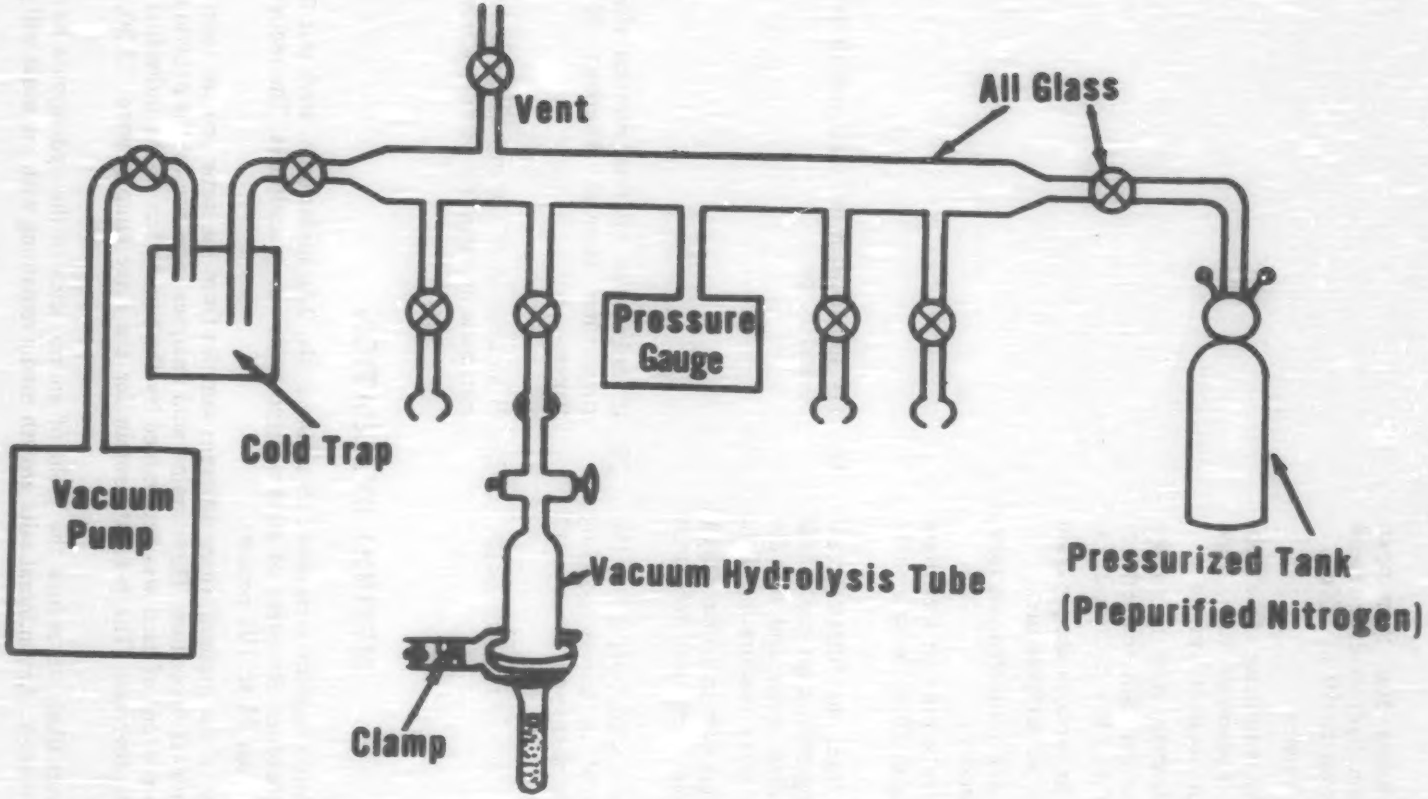

Vent
All Glass
Pressure Gauge
Cold Trap
Vacuum Pump
Vacuum Hydrolysis Tube
Clamp
Pressurized Tank
(Prepurified Nitrogen)

Figure 1. Pressure system for deaerating samples.

11. Evacuate system below 50μ, then open stopcock on vacuum hydrolysis tube and evacuate sample down to 50 to 100μ.	
12. Repeat steps 7 to 12 twice.	
13. While vacuum pump is running, close stopcock on vacuum hydrolysis tube, then remove tube from vacuum system.	
14. Place vacuum hydrolysis tube in an upright position in a constant temperature oven maintained at 110 C ± 1 C for 22 hr.	
15. At the end of 22 hr, remove sample from oven and cool to room temperature.	
16. Slowly open stopcock on hydrolysis tube, then remove top section.	
17. Filter contents of tube through a medium porosity, sintered glass filter under a slight vacuum.	
18. Pour the filtrate from the filtering flask into an evaporating dish; then wash flask 3 times with distilled water and transfer washings into the same evaporating dish.	18. Try to maintain the total volume between 20 and 30 cc.
19. Place the evaporating dish in a freeze drier and allow to remain there until residue is dry.	
20. Add 5 ml of citrate buffer, pH 2.2 to the freeze-dried material in the evaporating dish; stir with rubber policeman until residue is dissolved.	20. If undissolved material remains after stirring, filter through Whatman 40 filter paper.
21. Store hydrolyzate in a stoppered bottle.	21. If an analysis of the protein hydrolyzate is not made immediately, store in a freezer.

METHOD EVALUATION

Aliquots of a calibration mixture containing 18 amino acids, 0.5μ moles each, were put through the same hydrolysis procedure. Recovery of all the amino acids was quantitative. The recovery for all amino acids ranged from 95 to 101 percent.

To test the accuracy of the method, three different samples from the same protein source were hydrolyzed using the subject procedure. Three amino acid analyses were run on the protein hydrolyzates, and the protein content of each was determined by the summation of the individual amino acids present in each hydrolyzate. The percent protein for the three samples were: 13.30, 13.39, and 13.15.

The interferences most likely to reduce the yield of amino acids in the hydrolyzate have been pointed out in the Comments. Any mineral salts growth media remaining with the mold will greatly reduce the accuracy of an analysis. The presence of undigested starchy and cellulosic substrates in the hydrolysis mixture causes oxidation of the amino acids, thus producing lower yields.

The washing and drying process normally requires a 24-hr period, and another 24-hr are required for the degassing and hydrolysis process. The analyst could save a day's work if the laboratory submitting the protein sample were instructed as to the washing and drying techniques. The degassing and hydrolysis procedures could be started immediately if the sample were dried and ground on receipt.

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LABORATORY PROCEDURE FOR THE SPECTROPHOTOFLUOROMETRIC DETERMINATION OF SELENIUM IN SOLID WASTE[*]

Henry Johnson[†]

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[*] A description of this method appears in "Determination of Selenium in Solid Waste," Henry Johnson, Environmental Science & Technology, 4:850-853, Oct. 1970.

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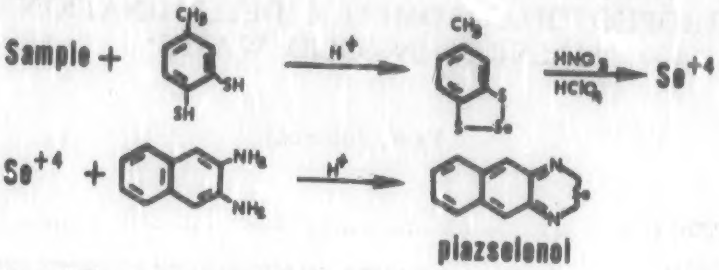
DISCUSSION

The determination of selenium in solid waste and in the effluents of solid waste treatment systems is of considerable interest because of the toxicity of selenium and its reported presence in almost all conceivable types of paper. A 2,3-diaminonaphthalene (DAN) analytical procedure is preceded by isolation of selenium with toluene 3, 4-dithiol and includes the use of EDTA, sodium fluoride, and sodium oxalate masking agents. The reactions in the procedure are thought to involve the formation of a piazselenol as follows:



The piazselenol emits at 570 mμ when excited at 317 mμ.

The use of the DAN fluorometric procedure affords the sensitivity necessary for trace analysis and at the same time retains a precision of measurement comparable to competitive methods. The isolation step and masking agents are used to prevent background fluorescence and foreign ion effect.

APPARATUS

The apparatus required for the analyses is:

1. Farrand Mark I Spectrofluorometer with 10mm slits and RCA IP 121 phototube
2. Wiley Mill fitted with a 10 mesh screen or a Williams patent hammermill and double-cone homogenizer.
3. Parr bomb calorimeter
4. Beckman zeromatic pH meter
5. Sorval SS-1 centrifuge

REAGENTS

The analysis requires the following reagent grade chemicals:

1. Hydrochloric acid
2. Sodium hydroxide
3. Nitric acid
4. 72 percent perchloric acid

5. Ethylene chloride
6. Carbon tetrachloride
7. Cyclohexane
8. 2,3-diaminonaphthalene (DAN) (Aldrich Chemical Co.)
9. Toluene-3,4-dithiol zinc salt (Eastman Organic Chemicals)
10. Sodium fluoride
11. Sodium oxalate ($Na_2C_2O_4$)
12. Sodium selenite (Na_2SeO_3)
13. Ethylenediaminetetraacetic acid disodium salt (EDTA)
14. Sulfuric acid
15. Quinine

Prepare solutions of the above reagents as follows:

1. Prepare 0.1 N HCl by diluting 8.3 ml of concentrated HCl to 1,000 ml with distilled water.
2. Prepare 0.1 N NaOH by dissolving 4.0 g of NaOH in 1,000 ml of distilled water.
3. Dissolve 480 g of NaOH in 1,000 ml of distilled water to produce an approximately 12 N solution.
4. Prepare 0.1 percent DAN fresh daily by dissolving 0.1 g of DAN in 100 ml of 0.1 N HCl.
5. Prepare a suitable dithiol solution fresh daily by adding 1 g of toluene-3,4-dithiol zinc salt to several milliliters of ethanol. Add several drops of concentrated HCl and heat the mixture under the tap to produce a clear solution. Finally, dilute the solution to 100 ml with ethanol.
6. Prepare 0.1 M sodium fluoride by dissolving 4.2 g in 1,000 ml of distilled water.
7. Prepare 0.1 M sodium oxalate by dissolving 13.4 g in 1,000 ml of distilled water.
8. Prepare 0.1 M EDTA by dissolving 37.2 g in 1,000 ml of distilled water.
9. Prepare a standard solution of selenium by dissolving 2.1881 g of Na_2SeO_3 in 1,000 ml of distilled water. The concentration of this solution is 1,000 μg Se per milliliter.
10. Dilute 2.8 ml of concentrated H_2SO_4 to 1,000 ml, thus producing a 0.1 N solution.
11. Prepare a stock standard of quinine sulfate by dissolving 0.0100 g of quinine in 1,000 ml of 0.1 N H_2SO_4 thus producing a concentration of 10 μg per milliliter. Prepare a working standard by diluting 1 ml of stock standard to 10 ml with 0.1 N H_2SO_4. These solutions should remain in the dark when not in use.

SAFETY PRECAUTIONS

Adequately ventilate the spectrofluorometer lamp housing (with a hood if possible) because ozone is produced when the instrument is in use.

Take extreme caution during the nitric-perchloric acid digestions. These should never be taken to dryness because salts of perchloric acid are explosive.

Follow the manufacturer's instructions when using the Parr bomb, and follow general laboratory safety practices at all times.

SAMPLE PREPARATION

Grind, mix, and dry refuse and compost as described in an earlier chapter. Pelletize 1-g samples and ash in a Parr bomb using 10 to 15 ml of water as an absorbing solution under 30 atm. of oxygen pressure. Tap cool the bomb for 10 min and release the excess oxygen over a 2-min period. Filter the bomb contents and washings through Whatman #12 filter paper and adjust the volume to 25 ml.

Place glass fiber millipore filters containing particulates collected by isokinetic stack sampling in a 100 ml beaker and add 15 ml of a 10:1 HNO_3 and $HClO_4$ acid mixture. Heat the mixture cautiously to remove oxides of nitrogen. After adding 25 ml of water, bring the solution to a boil, allow to cool, and pass through a sintered glass filter. Adjust the volume to 50 ml. Extract samples (1 g) of incinerator residue in the same manner.

PROCEDURE

1. Transfer 25 ml or an aliquot containing not less than 0.01 μg of Se into a 125-ml separatory funnel, adjust the volume to 35 ml, and add 50 ml of concentrated HCl.
2. Add 4 ml of freshly prepared 1 percent zinc dithiol suspension in ethanol. Mix and let stand 15 min.
3. Extract consecutively with a 10 ml and 5 ml mixture of ethylene chloride and carbon tetrachloride (1:1) and combine the organic phases in a stoppered test tube.
4. Add 1 ml of 72 percent $HClO_4$ and 10 drops of concentrated HNO_3 and boil off organic solvent in boiling water bath.
5. Heat cautiously until fumes of $HClO_4$ are in evidence. Add 1 ml of H_2O, heat until fumes of $HClO_4$ are apparent again, and add 10 ml of H_2O.
6. Adjust the pH to 2 with NaOH and add 0.5 ml each of 0.1 M aqueous solutions of EDTA, NaF, and $Na_2C_2O_4$.
7. Readjust the pH to 2 with HCl and add 4 ml of freshly prepared 0.1 percent DAN in 0.1 N HCl.
8. Place the tubes in a 50 C H_2O bath for 20 min and tap cool.
9. Transfer contents of tubes to 125-ml separatory funnels containing 10 ml of cyclohexane. Extract and collect organic phase in a centrifuge tube.
10. Centrifuge at 2,000 rpm for 2 min and read on spectrofluorometer exciting at 370 mμ and emitting 517 mμ.

STANDARDIZATION AND CALIBRATION

Prepare a calibration curve by analyzing standard solutions ranging from 0.005 to 1.0 μg Se. A linear relationship should be obtained by plotting the concentration of selenium versus the fluorescent intensity. A distilled water blank should give negligible readings at the analytical wave lengths. Bracket each sample reading by readings of 1 μg per milliliter quinine sulfate solution to account for fluctuations in lamp intensity.

CALCULATIONS

The formula needed to calculate the concentration of selenium in samples is:

$$C = (MM \times T_s) \div (S)\left(\frac{MM \times T_{Qc}}{MM \times T_{Qs}}\right)\left(\frac{10}{V_s}\right)$$

where

C = concentration of selenium in 10 ml cyclohexane
MM = meter multiplier setting
T_s = percent transmittance of the sample
S = slope of the calibration curve
T_{Qc} = percent transmittance of quinine for the calibration curve
T_{Qs} = percent transmittance of quinine for the sample run
V_s = volume of sample analyzed

METHOD EVALUATION

Eleven samples of standard solution containing 1 μg of selenium per 10 ml of test solution were analyzed over a 2-day period. The results from these runs gave a standard deviation of 0.078 and a relative error of 2.57 percent. The standard deviation indicates ± 0.03 μg of selenium for the analyses of 1 μg standard solutions.

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PART III

MICROBIOLOGICAL METHODS

METHODS FOR BACTERIOLOGICAL EXAMINATION OF SOLID WASTE AND WASTE EFFLUENTS

Mirdza L. Peterson*

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INTRODUCTION AND GENERAL LABORATORY PROCEDURES

Introduction

Developing methods to detect and enumerate bacterial pathogens in solid waste and waste effluents, particularly those found in fecal and food waste, is an important microbiological research goal of this Solid Waste Research Laboratory (1). Attempts to isolate such organisms from solid waste on a routine basis have not been fruitful because of low initial numbers and/or relatively short periods of survival. Pathogenic microorganisms in waste are constantly subjected to such debilitating environmental factors as chemical additives, drying, freezing, heat, and pH extremes. These factors often affect cultivation of these organisms in media originally designed for diagnostic purposes. For these reasons, attention was directed primarily toward the development of methods for the detection and enumeration of a group of organisms that are of significance in the fields of public health and sanitation. Three procedural lines of investigation were undertaken:

1. To develop suitable methods for indicating the sanitary quality of solid waste before and after processing or disposal.
2. To develop suitable methods for determining the efficacy of operational procedures for removing or destroying the microorganisms.
3. To develop suitable methods for indicating the health hazard of solid waste in which pathogenic agents may be present in small numbers.

An investigation was made to evaluate presently employed bacteriological methods applicable to solid waste and related materials. This evaluation led to the establishment of reliable methods that are well-suited to routinely measuring, under practical conditions, the bacteriological quality of solid waste, incinerator residue, industrial and quench waters, leachate, stack emissions, and dust in and around waste processing areas. The methods described here will determine:

1. Total number of viable bacterial cells
2. Total coliforms
3. Fecal coliforms
4. Heat-resistant spores
5. Enteric pathogens, especially Salmonella sp.

It should be noted that minor changes in technical procedure may result in marked changes in the validity of the data.

General Laboratory Procedures

Glassware washing.

All glassware known to contain infectious material must be sterilized by autoclaving before washing. All glassware that is to be used in microbiological tests must be thoroughly washed before sterilization, using a suitable detergent and hot water, and followed by hot water and distilled water rinses. Six to 12 rinses may be required to remove all traces of inhibitory residues from the glass surface.

Sterilization.

Dry heat is used for the sterilization of glass sampling bottles, foil-covered flasks, beakers, graduates, pipettes packed tightly in sealed cans, or articles that are corrosively attacked be steam. Recommended time-temperature ratio for dry heat sterilization is 170 C for 2 hr.

Saturated steam under pressure (or autoclaving) is the most frequently used sterilization method. Media, dilution water, and materials (rubber, paper, cotton, cork, heat-stable plastic tubes, and closures, for example) are sterilized by autoclaving at 121 C. Sterilization time for media and dilution water (for volumes up to 500 ml) is 15 min; 1,000-ml quantities are held for 20 min, instruments for 15 min, gloves for 20 min, and packs for 30 min (measured from the time the autoclave temperature reaches 121 C).

Membrane filters are sterilized for 10 min at 121 C with fast steam exhaust at the end of the sterilization process.

Heat-sensitive carbohydrates and other compounds are sterilized by passage through a cellulose esther membrane or another bacteria-retaining filter.

Culture media.

The use of dehydrated media is recommended whenever possible, since these products offer the advantages of good consistency from lot to lot, require less labor in preparation, and are more economical. Each lot should be tested for performance before use.

Measurement of the final pH of a prepared culture medium should be accomplished colorimetrically after autoclaving and cooling. Acceptable pH range is 7.0 ± 0.1.

Media should be stored in a cool, dry, and dark place to avoid dehydration, deterioration, and adverse light effects. Storage in the refrigerator usually prolongs the shelf-life of most media. Media should not be subjected to long periods of storage, because certain chemical reactions may occur in a medium even at refrigerator temperatures.

Many of the media referred to below can be obtained from commercial sources in a dehydrated form with complete information on their preparation. These media will therefore be listed but not described in this section. Described in this section are those media that are formulated from ingredients or from dehydrated materials. Culture media (Difco or BBL products) are listed as follows:

Bacto-agar
Bismuth sulfite agar
Blood agar
Brain heart infusion broth
Brilliant green agar
Brilliant green lactose bile, 2 percent
Coagulase mannitol agar
Dextrose
E. C. broth
Eosin methylene blue agar, Levine
Fluid thioglycollate medium
Gelatin
H-broth
Indole nitrite medium
KCN medium

Lactose
Lactose tryptose broth
Lauryl tryptose broth
Lysine decarboxylase medium
M-Endo broth
M-FC broth
MacConkey's agar
Malonate broth, Ewing modified
Maltose
Mannitol
Mannitol salt agar
Methyl red-Voges Proskauer medium
Nitrate broth
Nutrient agar
Phenol red broth base
Phosphate buffer, APHA, pH 7.2
Sabouraud's dextrose agar
Salmonella-Shigella agar
SBG enrichment broth
Selenite-F enrichment broth
SIM medium
Simmons citrate agar
Sucrose
Triple sugar iron agar
Trypticase soy agar
Tryptone glucose extract agar
Urea agar base concentrate (sterile)
XLD agar

Culture media requiring preparation.

1. Blood Agar: Suspend 40 g of trypticase soy agar in a liter of distilled water. Mix thoroughly. Heat with agitation and boil for 1 min. After solution is accomplished, sterilize by autoclaving for 15 min at 121 C. Cool agar to 45 to 50 C, and add 5 to 7 percent sterile, defibrinated sheep blood, mixing evenly throughout the medium. Pour into sterile Petri dishes. After solidification, invert dishes and incubate overnite.
2. Phenol Red Broth Base: Dissolve 15 g in a liter of distilled water. Add 5 to 10 g of desired carbohydrate. Use Durham fermentation tubes for detection of gas formation. Arrange tubes loosely in suitable containers and sterilize at 116 to 118 C for 15 min.
3. Phosphate Buffer Solution: To prepare stock phosphate buffer solution, dissolve 34.0 g potassium dihydrogen phosphate, KH_2PO_4, in 500 ml distilled water, adjust to pH 7.2 with 1N NaOH, and dilute to 1 liter with distilled water. Add 1.25 ml stock phosphate buffer solution to 1 liter distilled water. Dispense in amounts that will provide 99 ± 2.0 ml or 9 ± 0.2 ml after autoclaving at 121 C for 15 min.

COLLECTION AND PREPARATION OF SAMPLES

Method for Collection of Solid Waste or Semi-Solid Waste Samples

Equipment and materials.

Necessary items are as follows:

1. Sample containers, specimen cups, sterile, 200-ml size (Falcon Plastics, Los Angeles)
2. Sampling tongs, sterile (stainless steel, angled tips, 18 in. long)
3. Shipping container, insulated, refrigerated, 6 by 12 in. I.D.
4. Disposable gloves

Procedure.

1. Using sterile tongs, collect 20 to 40 random 100- to 200-g samples and place in sterile sampling containers. When collecting samples from contaminated sources, wear disposable gloves and avoid contaminating the outside of the container.

2. Identify samples on tag and indicate time and date of sampling. If incinerator residue samples are taken, record operating temperatures of incinerator.

3. Deliver samples to laboratory. It is recommended that the examination be started preferably within 1 hr after collection;* the time elapsing between collection and examination should in no case exceed 8 hr.

Method for Collection of Liquid Samples-Quench and Industrial Waters or Leachate

Equipment and materials.

Necessary items include a screw-capped, 250-ml, sterile sample bottle or a 16-oz, sterile plastic bag.

Procedure.

Collect sample in bottle or plastic bag, leaving an air space in the container to facilitate mixing of the sample before examination. When collecting samples from contaminated sources, wear disposable gloves and avoid contaminating the outside of the container.

Identify and deliver samples to laboratory. When shipping samples to laboratory, protect containers from crushing and maintain temperature below 10C during a maximum transport time of 6 hr. Examine within 2 hr. If water sample contains residual chlorine, a dechlorination agent such as sodium thiosulfate is added to collection bottles to neutralize any residual chlorine and to prevent a continuation of the bactericidal action of chlorine during the time the sample is in transit to the laboratory. Enough sodium thiosulfate is added to the clean sample bottle before sterilization to provide an approximate concentration of 100 mg per liter in the sample.

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*If sample is shipped to a laboratory for analysis and examination cannot begin within 1 hr of collection, the container must be insulated and sample maintained below 10 C during the maximum transport of 6 hr. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hr.

Method for Collection of Incinerator Stack Effluents

Equipment and materials.

Necessary items include an Armstrong portable sampler (2), equipped with sampling assembly (Figure 1). The sampler is mounted on a steel plate (6 by 12 in.) and can be enclosed by a metal cover with a handle attached. On one side of the base is a vacuum pump with a 6-ft cord and switch. The pump is capable of drawing up to 1 cu ft per min of air (vacuum of 5.6 in. [14.3 cm] of water). On the other side of the base, a 700-ml, wide-mouth, Pyrex bottle contains 300 ml of 0.067 M phosphate buffer solution (pH 7.2) prepared by standard methods (3). The two-hole rubber stopper has a 1-in. (2.54 cm) piece of cotton-plugged glass tubing in one of the two holes. The stopper, glass tube, and contents of the bottle are maintained sterile. The bottle is held to the base plate by three removable spring clips, which are attached at the base and at a wire triangle slipped over the top of the bottle. The sampling probe is made of stainless steel tubing of appropriate diameter (e.g., 0.25-in. I.D. [0.64 cm]). The probe end has a right-angle bend so that the opening faces the stack-gas current. The tubing must be long enough to reach all parts of the stack. The tubing is coiled to permit additional cooling of the gases and is straight for 1 or 2 ft (30.48 or 60.96 cm) at a right angle to the other straight length. Before use, the sampling probe is sterilized by dry heat sterilization. It is important to keep the inside of the probe dry to minimize adsorption of microorganisms on the walls of the tubing. When sampling, the probe is inserted into the stack at locations that will yield a representative sample. The other end of the sterile probe is inserted through the sterile rubber stopper to approximately 0.5 in. (1.27 cm) above the buffered water. This is done to reduce the frothing that would occur if the probe were inserted below the surface; enough froth results in capturing the microorganisms.

Procedure.

1. Draw stack effluent through the sterile stainless steel tube by a 1.0 cfm vacuum pump; cool the tube with a water jacket.
2. Obtain a 10-cu-ft sample by drawing the stack effluent for 10 min.
3. Identify sample on tag and examine within 4 hr. The Armstrong portable sampler provides a method for qualitative, nonisokinetic sampling and is adjustable to isokinetic conditions.

Method for Collection of Dust Samples

Equipment and materials.

Necessary items include the following:

1. Andersen sampler (4)
2. Trypticase soy agar containing 5 percent sheep blood (6 plates per sample)
3. Eosin methylene blue agar

Procedure.

1. Draw air through the sterile, assembled sampler at 1.0 cfm with a vacuum of 15 in. of mercury.
2. Remove agar plates from the sampler, cover, and incubate at 35 ± 0.5 C. Use aseptic technique throughout the procedure.

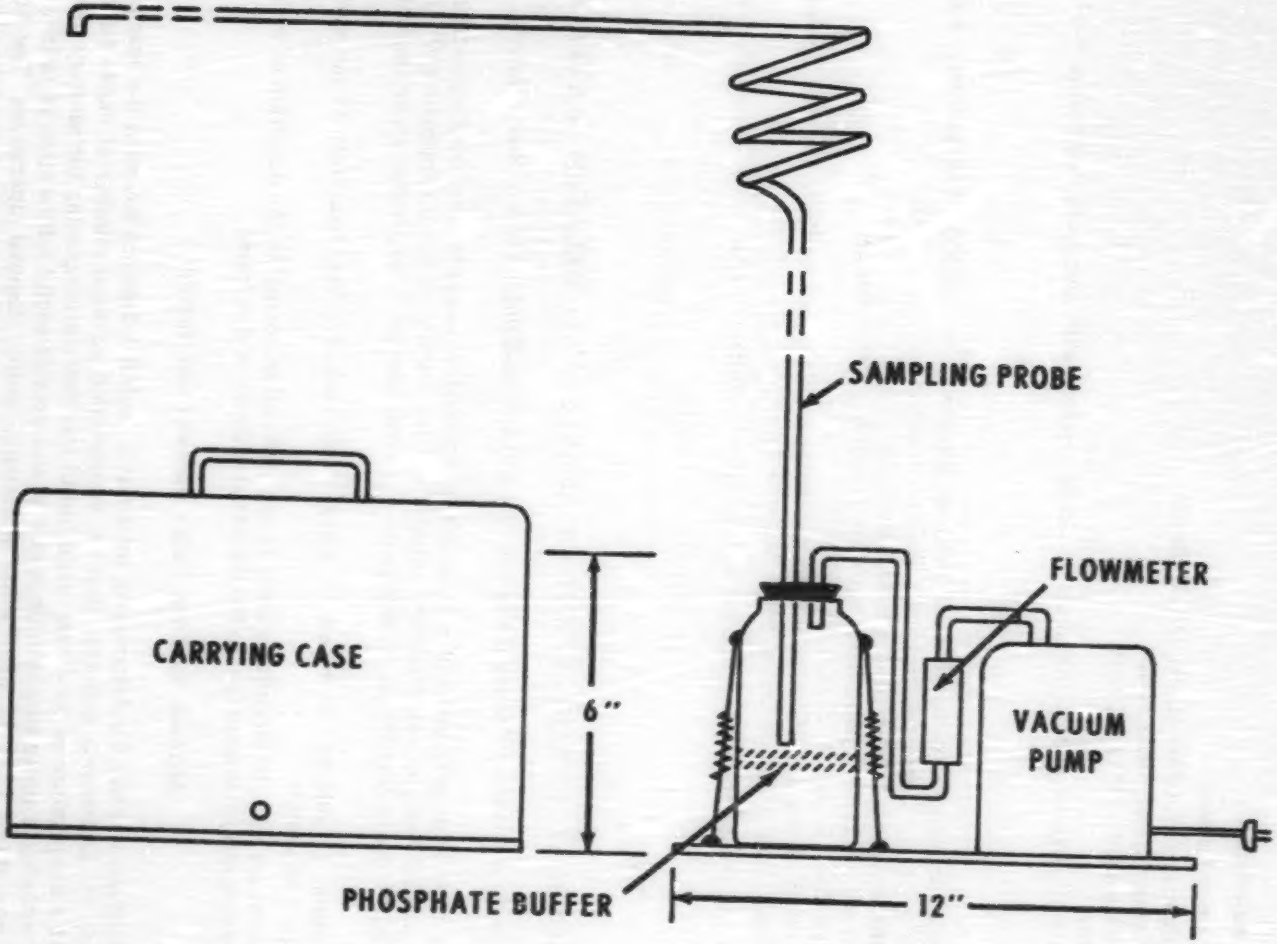
SAMPLING PROBE
FLOWMETER
CARRYING CASE
VACUUM PUMP
6"
PHOSPHATE BUFFER
12"

Figure 1. Portable sampler for microorganisms in incinerator stack emission.

Method for Preparation of Solid and Semi-Solid Samples for Analyses

Equipment and materials.

Necessary items are as follows:

1. Cold phosphate buffer, 0.067 M, pH 7.2, sterile (3)
2. Blender, Waring (Model 1088), sterile
3. Balance, with weights, 500-g capacity
4. Tongs, sterile
5. Beakers, two, 5,000 ml and 1,000-ml sizes, sterile, covered with aluminum foil before sterilization.

Procedure.

1. Using aseptic technique, composite all random samples into a 5,000-ml beaker. Mix well.
2. Weigh 200 g of the subsample into a 1,000-ml beaker.
3. Transfer the weighed sample to a sterile blender.
4. Add 1,800 ml of sterile, phosphate buffered solution to the blender.
5. Homogenize for 15 sec at 17,000 rpm (5).
6. Prepare a series of decimal dilutions as described below in "Methods for Preparation of Decimal Dilutions of a Solid, Semi-Solid, or Liquid Waste Material."

Solid waste and residue samples for enteric pathogenic bacteria are examined directly without homogenization.

BACTERIOLOGICAL EXAMINATION OF WASTE AND RELATED MATERIALS

Method for Preparation of Decimal Dilutions of a Solid, Semi-Solid, or Liquid Waste Material

Immediately after homogenization of any sample (see procedure under Method for Preparation of Solid and Semi-Solid Samples for Analyses) transfer a 1-ml portion of the homogenate (10^{-1} dil) to a dilution bottle containing 99 ml of phosphate buffered solution. Stopper and shake the bottle 25 times.

Prepare dilutions as indicated in Figure 2. Again shake each dilution vigorously 25 times after adding an aliquot of sample.

These dilutions are used to inoculate a series of selected culture media for the detection of various groups of microorganisms as described in the following sections of this paper.

Methods for Total Viable Bacterial Cell Number

The chief cultural method for determining total viable bacterial densities has been the agar plate method (3, 6, 7). Experience indicates that an enumeration of total number of viable bacteria multiplying at a temperature of 35 C may yield useful information concerning the sanitary quality of the waste entering a processing or a disposal site and provide useful information in judging the efficiency of procedures used in solid waste processing and/or disposal operations. The viable microbial count also provides valuable information concerning the microbiological quality of environmental aerosols existing in or around a waste processing plant or a disposal site.

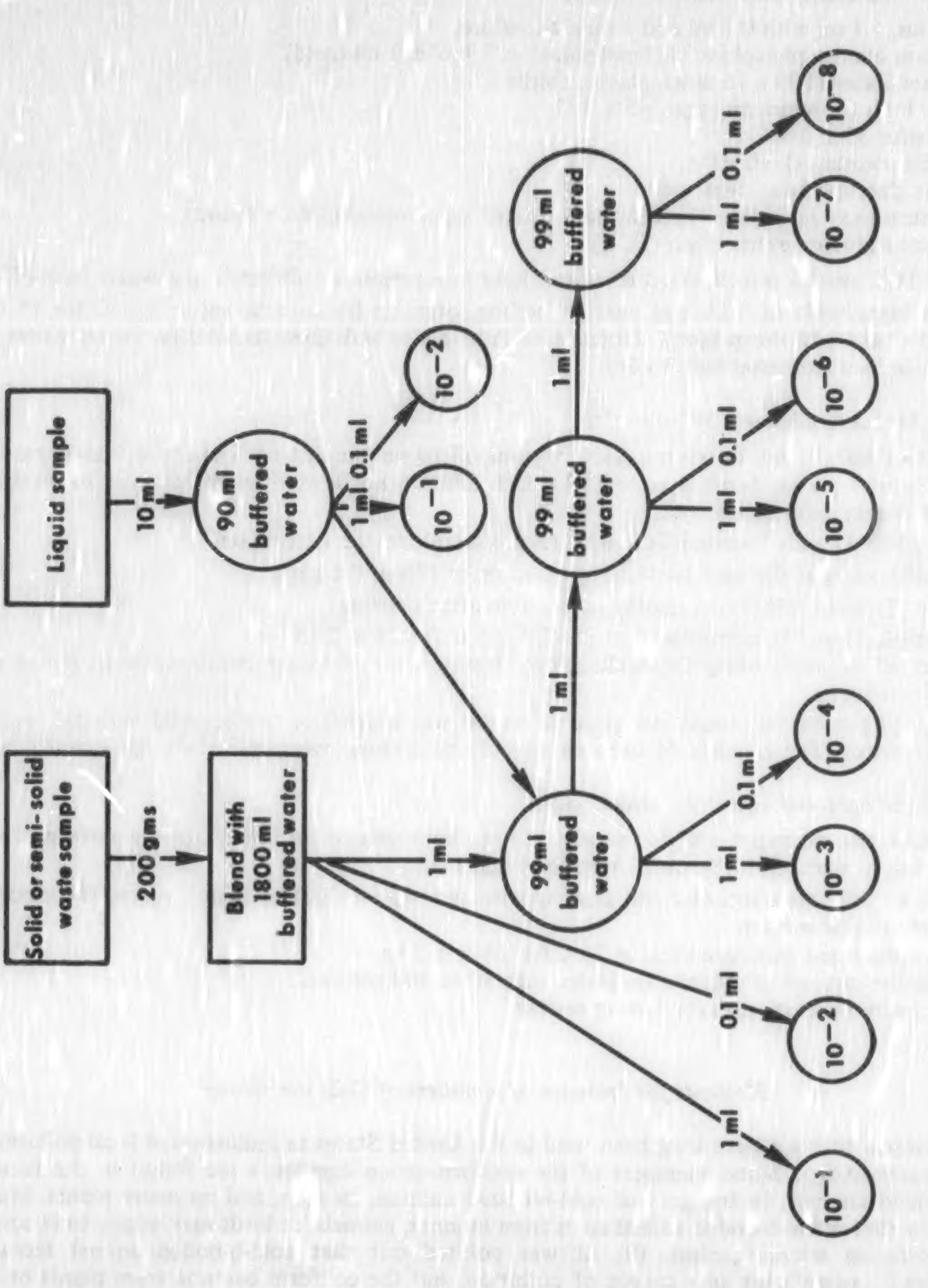
Solid or semi-solid waste sample
200 gms
Blend with 1800 ml buffered water
Liquid sample
10 ml
90 ml buffered water
1 ml
0.1 ml
10^{-1}
10^{-2}
99ml buffered water
1 ml
0.1 ml
10^{-1}
10^{-2}
10^{-3}
10^{-4}
99 ml buffered water
10^{-5}
10^{-6}
99 ml buffered water
10^{-7}
10^{-8}

Figure 2. Preparation of decimal dilutions.

Equipment, materials, and culture media.

1. Pipettes, 1.1 ml with 0.1 ml and 1 ml graduations
2. Dilution blanks, phosphate buffered solution, 99 ml ± 1 ml (cold)
3. Culture dishes (100 x 15 mm), plastic, sterile
4. Water bath for tempering agar, 45 ± 1 C
5. Incubator 35 ± 0.5 C
6. Colony counter, Quebeck
7. Sterile glass spreader, bent rod
8. Trypticase soy agar with 7 percent defibrinated sheep blood (TSA + blood)
9. Tryptone glucose extract agar (TGE)

Prepare TGE agar as indicated on label and hold in a melted condition in the water bath (45 C).

Dissolve ingredients of TSA and heat to boiling. Sterilize by autoclaving at 121 C for 15 min. Cool to 45 C and add sheep blood. Dispense in Petri plates and allow to solidify. Invert plates and place them in incubator overnight to dry.

Procedure for bacterial count by pour plate.

1. Pipette 1 ml, 0.1 ml, or other suitable volume of the sample into each of appropriately marked, duplicate culture plates, being sure to shake each dilution bottle vigorously 25 times to resuspend material that may have settled out.
2. Add 10 to 12 ml of melted TGE agar to the sample in the Petri plate.
3. Mix dilution and the agar medium by rotating or tilting the plate.
4. Allow plates to solidify as rapidly as possible after pouring.
5. Invert plates and incubate them at 35 C ± 0.5 C for 24 ± 2 hr.
6. Count all colonies using Quebeck colony counter, the objective being to count plates with 30 to 300 colonies.
7. Compute the colony count per gram of waste (wet weight) or related solid material, and per 100 ml of water. The number of bacteria should not include more than two significant figures.

Procedure for bacterial count by streak plate.

1. Dispense 0.1 ml samples of the serially diluted homogenate (or liquid) on the surface of each of appropriately marked, duplicate TSA + blood agar plates.
2. Using a sterile glass spreader and starting with the highest dilution plates, spread the inoculum evenly over the agar surface.
3. Invert plates and incubate them at 35 C for 24 hr ± 2 hr.
4. Count the number of colonies on plates with 30 to 300 colonies.
5. Select and mark colonies for further testing.

Methods for Presence of Members of Coliform Group

The coliform bacteria have long been used in the United States as indicators of fecal pollution in sanitary bacteriology. Some members of the coliform-group organisms are found in the feces of warm-blooded animals, in the guts of cold-blooded animals, in soils, and on many plants. Studies have shown that warm-blooded animal feces from humans, animals, or birds may at any time contain disease-producing microorganisms (8). It was pointed out that cold-blooded animal feces are quantitatively insignificant as a source of pollution, but the coliform bacteria from plants or soils

that have been recently exposed to fecal pollution have the same significance as those from feces. On the other hand, the coliform bacteria deriving from soils or plants that have not been exposed to recent fecal contamination has less public health significance.

Adequate treatment of waste before disposal and proper operational design of a waste processing plant should remove all coliform organisms. Treated or processed waste containing coliform bacteria demonstrates an inadequate treatment and should be considered of sanitary significance. The contamination of waste by fecal matter may be one avenue of transmission of pathogenic microorganisms to the environment and man.

The presence of fecal matter in waste and related materials is determined by the standard tests for the coliform group described in Standard Methods for the Examination of Water and Waste Water (3). The completed Most Probable Number (MPN) procedure is employed. The testing method includes the elevated temperature test (44.5 C) that indicates the fecal or nonfecal origin of coliform bacteria. Comparative laboratory studies conducted showed that the MPN estimate is the most suitable method for achieving a representative enumeration of the coliform organisms in solid waste and waste effluents (9).

Equipment and materials.

1. **Pipettes, sterile–deliveries to 10 ml, 1 ml (1.1 ml), and 0.1 ml**
2. **Media prepared in fermentation tubes:**
   Lauryl tryptose broth
   Brilliant green lactose bile broth, 2 percent
   Lactose tryptose broth
   E.C. broth
3. **Media for plating:**
   Eosin methylene blue agar plates
   Nutrient agar slants
4. **Dilution blanks, phosphate buffer solution, sterile, 99-ml or 90-ml amounts**
5. **Incubator, adjusted to 35 C ± 0.5 C**
6. **Water bath, adjusted to 44.5 C ± 0.2 C**

Procedure for total coliform group.

Presumptive Test.

1. Inoculate a predetermined volume of sample into each of 5 lauryl tryptose broth tubes. The portions of the sample used for inoculation should be decimal multiples and submultiples of 1 ml.

2. Incubate the fermentation tubes at 35 ± 0.5 C for 24 ± 2 hr.

3. Examine for the presence of gas. If no gas is formed, incubate up to 48 ± 3 hr. Record the presence or absence of gas formation at each examination of the tubes, regardless of the amount.

Confirmed Test.

1. Submit all presumptive test tubes showing any amount of gas at the end of 24- and 48-hr incubation to the confirmed test. Using a sterile platinum loop 3 mm in diameter, transfer one loopful of medium from the presumptive test fermentation tube to a fermentation tube containing brilliant green lactose bile broth.

2. Incubate the inoculated brilliant green lactose bile broth tube for 48 ±3 hr at 35 ± 0.5 C. The presence of gas in any amount in the fermentation tube of the brilliant green lactose bile broth within 48 ± 3 hr indicates a positive confirmed test.

Completed Test.

1. Submit all confirmed test tubes showing any amount of gas to the completed test. Streak an eosin methylene blue agar plate from each brilliant green bile broth tube as soon as possible after the appearance of gas.
2. Incubate the plates at 35 ± 0.5 C for 24 ± 2 hr.
3. Fish one or more typical or atypical colonies from plating medium to lactose tryptose broth fermentation tubes and nutrient agar slants.
4. Incubate the broth tubes and the agar slants at 35 ± 0.5 C for 24 ± 2 hr or 48 ± 3 hr if gas is not produced in 24 hr.
5. Prepare gram stained smears from the nutrient agar slants if gas is produced in any amount from lactose broth.
6. Examine smears under oil immersion. If typical coliform staining and morphology are found on the slant, the test may be considered completed and the presence of coliform organisms demonstrated.

Procedure for fecal coliform group (E. C. broth).

1. Submit all gas positive tubes from the Standard Methods presumptive test (lauryl tryptose broth) to the fecal coliform test. Inoculate an E. C. broth fermentation tube with a 3-mm loop of broth from a positive presumptive tube.
2. Incubate the broth tube in a water bath at 44.5 ± 0.2 C for 24 hr. All E. C. tubes must be placed in the water bath within 30 min after planting.
3. Gas production in the E. C. broth fermentation tubes within 24 hr ± 2 hr is considered a positive reaction indicating fecal origin.

Computing and recording most probable number (MPN).

The calculated estimate and the 95 percent confidence limits of the MPN described in the 13th edition of Standards Methods for Examination of Water and Waste Water (3) are presented in Table 1. This table is based on five 10-ml, five 1.0-ml, and five 0.1-ml sample portions. When the series of decimal dilutions such as 1.0, 0.1, and 0.01 ml are planted, record 10 times the value in the table; if a combination of portions of 0.1, 0.01, and 0.001 ml are planted, record 100 times the value in the table. MPN values for solid samples are calculated per g of wet weight; MPN for liquid samples are recorded per 100 ml.

Method to Determine the Presence of Viable Heat-Resistant Spore Number

It is important to enumerate those heat-resistant, spore-forming microorganisms in waste, incinerator residue, and quench or industrial waters that survive a temperature of 80 C for as long as 30 min. With respect to mere survival of heat, most microorganisms in an actively growing (vegetative) state are readily killed by exposures to temperatures of about 70 C for 1 to 5 min (10). Cells inside solid material such as discarded meat products may escape heat longer because the heat does not penetrate immediately to the center of solid masses. Large masses of nonfluid solid matter require a long time (1½ to 2 hr), even in the autoclave (121 C), to be heated thoroughly enough for the center to reach a sporocidal temperature. Other reports point out (11) that although internal air temperatures of municipal incinerators usually range from 1,200 to 1,700 F (650 to 925 C) in continuous operation, intermittent use and overcharging of the incinerator and moisture content of the waste may interfere with sterilization of the residue.

TABLE 1.
MPN INDEX AND 95 PERCENT CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS, FIVE 1-ML PORTIONS, AND FIVE 0.1-ML PORTIONS ARE USED.*

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence Limits	
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each		Lower	Upper
0	0	0	<2		
0	0	1	2	<0.5	7
0	1	0	2	<0.5	7
0	2	0	4	<0.5	11
1	0	0	2	<0.5	7
1	0	1	4	<0.5	11
1	1	0	4	<0.5	11
1	1	1	6	<0.5	15
1	2	0	6	<0.5	15
2	0	0	5	<0.5	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500
5	4	0	130	35	300
5	4	1	170	43	490
5	4	2	220	57	700
5	4	3	280	90	850
5	4	4	350	120	1,000
5	5	0	240	68	750
5	5	1	350	120	1,000
5	5	2	540	180	1,400
5	5	3	920	300	3,200
5	5	4	1600	640	5,800
5	5	5	≥2400		

*Source: *Standard Methods for the Examination of Water and Wastewater.* 13th ed. Published, 1971, p. 673. Reproduced by permission, American Public Health Association, American Water Works Association, and Water Pollution Control Federation.

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A test of this type reveals operational problems of a waste processing plant and identifies the unsatisfactory quality of waste effluents of a municipal incinerator.

Equipment and materials.

1. Test tubes, sterile, screw capped, 20 x 150 mm
2. Pipettes, sterile, graduated, 10-ml
3. Water bath, electrically heated, thermostatically controlled at 80 ± 0.5 C, equipped with thermometer (range 0 to 110 C), NBS certified. Volume of water should be sufficient to absorb cooling effect of rack of tubes without drop in temperature greater than 0.5 C.
4. Test tube support for holding tubes

Procedure.

1. Transfer 10 ml from each original sample and from each successive dilution thereof to screw-capped test tubes, being careful to avoid contaminating the lip and upper portion of tube with sample.
2. Place tubes in a rack.
3. Place rack of tubes in water bath at 80 C for 30 min. Tubes should be immersed so that the water line is approximately 1½ in. above the level of samples in the tubes.
4. At the end of the 30-min holding period, remove the rack of tubes from the water bath and place in cold water for 5 min to cool.
5. Determine viable heat-resistant spore count by agar pour-plate method (see, Procedure for Bacterial Count by Pour Plate *under* Methods for Total Viable Bacterial Cell Number).
6. Report results as "viable heat-resistant spore count per gram."

Methods to Detect Enteric Pathogenic Bacteria

Fecal pollution in the environment because of untreated and improperly disposed of waste may add enteric pathogenic bacteria to a body of water or a water supply. The most common type of pathogen that may be found in untreated waste is *Salmonella.* The wide distribution of the many types of salmonellae in many species of animals with which man has contact or may use as food makes it difficult to prevent transmission to man (12). Infections may occur through food, milk, or water contaminated with infected feces or urine, or by the actual ingestion of the infected animal tissues (13). Salmonella has been found in many water supplies (14), polluted waters (15-17), raw municipal refuse, and in incinerator residue (18, 19).

The detection of enteric pathogenic bacteria such as salmonellae and shigellae in municipal solid waste before and after a treatment and/or disposal determines the microbiological quality of the material and serves as a procedure to determine the efficacy of a waste treatment process in removing or destroying the waste-borne pathogens. Results obtained in the testing may also be used for the design of epidemiological studies in other programs.

The method described below has been tested in the field and has been described by Peterson and Klee (18) and Spino (19), using incubation temperatures of 39.5 C and 41.5 C.

Equipment, materials and media.

1. Incubator, 37 C
2. Water baths, constant temperature, 39.5 C and 41.5 C

3. Flasks, wide-mouth, 500-ml
4. Membrane filter holder
5. Flasks, vacuum, 2,000-ml
6. Balance, with weights, 100-g capacity
7. Needle, inoculating
8. Media and reagents:
    - Selenite brilliant green/sulfa enrichment broth
    - Selenite F enrichment broth
    - Eosin methylene blue (EMB) agar
    - Salmonella-Shigella (SS) agar
    - Bismuth sulfite (BS) agar
    - McConkey's agar
    - Brilliant green (BG) agar
    - Triple sugar iron (TSI) agar
    - Urea medium
    - XLD agar
    - Salmonella antiserums
    - Shigella antiserums
    - Biochemical media (15)
9. Diatomaceous earth (Johns-Manville, Celite 505), sterile

Procedure to detect pathogens in solid waste and incinerator residue.

1. Add a previously weighed, 30-g sample to each of two flasks containing 270 ml Selenite F enrichment broth, and also to each of two flasks containing 270 ml Selenite brilliant green/sulfa (SBG) enrichment broth. Shake to mix.
2. Incubate one Selenite F and one SBG flask at 39.5 C and the other two at 41.5 C for 16 to 18 hr.
3. After incubation, streak one loopful from each enrichment medium on each of four plates of Salmonella-Shigella and other selective enteric media.
4. Incubate the plates at 37 C for 24 to 48 hr and pick suspicious colonies to triple sugar iron agar slants.
5. Incubate the slants at 37 C for 24 hr and complete identification by appropriate methods as described by Edwards and Ewing (20). Isolation, preliminary identification, and biochemical testing are described in Figure 3 and in Table 2.

Procedure to detect pathogens in quench or industrial waters and in leachate.

1. Place enough sterile diatomaceous earth on the screen of a stainless steel membrane filter holder to form a 1-in. layer.
2. Filter 800-ml sample through the earth layer.
3. Remove one-half the diatomaceous earth layer with a sterile spatula and place into 90 ml of Selenite F enrichment broth; place other half of the earth layer into 90 ml of Selenite brilliant green/sulfa enrichment broth. Shake both flasks to mix.
4. Incubate both flasks in a water bath at 39.5 C for 16 to 18 hr.
5. Proceed as directed in steps 3 through 5 of Procedure to Select Pathogens in Solid Waste and Incinerator Residue.

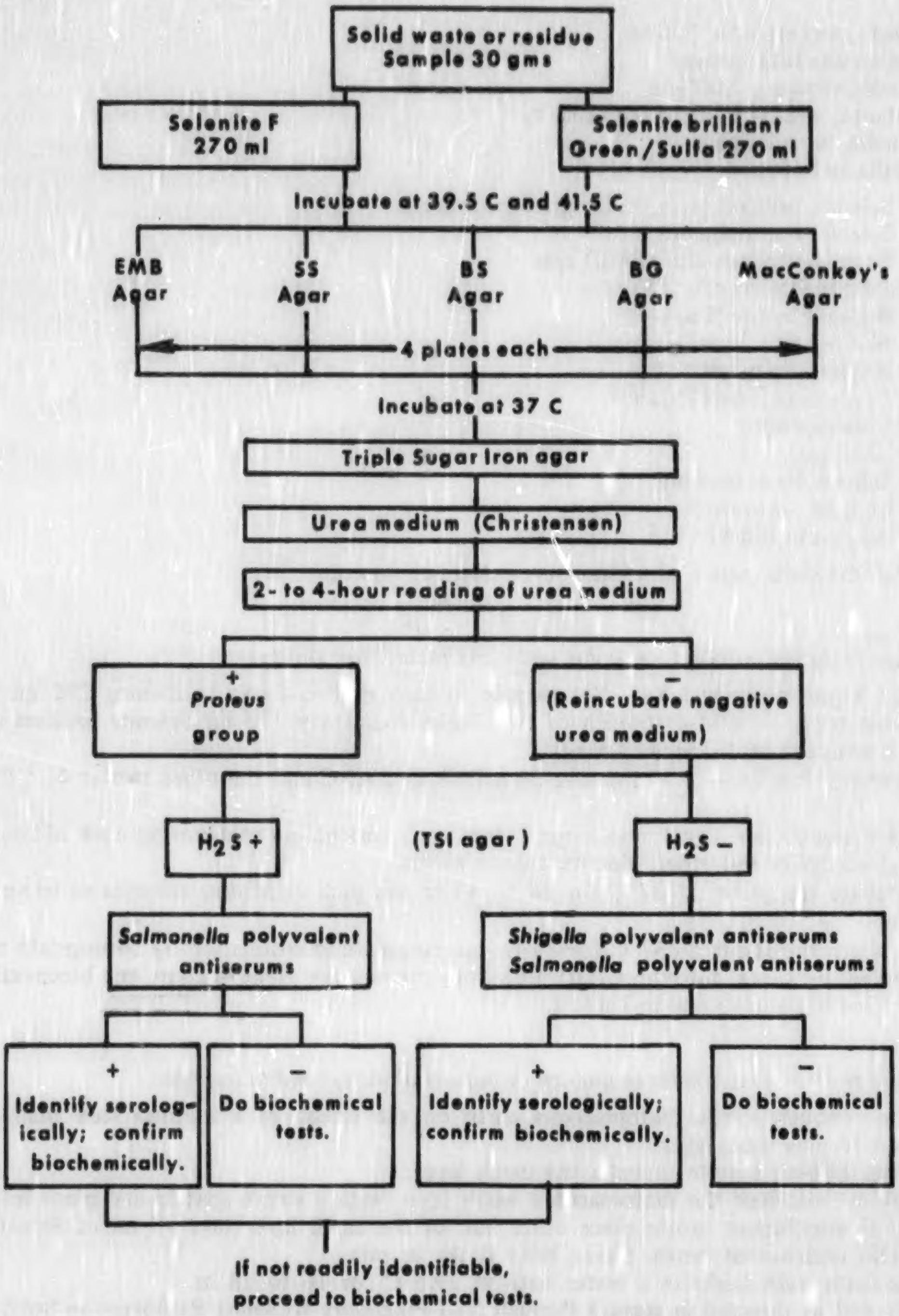
Solid waste or residue
Sample 30 gms
Selenite F
270 ml
Selenite brilliant
Green/Sulfa 270 ml
Incubate at 39.5 C and 41.5 C
EMB
Agar
SS
Agar
BS
Agar
BG
Agar
MacConkey's
Agar
4 plates each
Incubate at 37 C
Triple Sugar Iron agar
Urea medium (Christensen)
2- to 4-hour reading of urea medium
+
Proteus
group
–
(Reincubate negative
urea medium)
H_2S +
(TSI agar)
H_2S –
Salmonella polyvalent
antiserums
Shigella polyvalent antiserums
Salmonella polyvalent antiserums
+
Identify serologically; confirm
biochemically.
–
Do biochemical
tests.
+
Identify serologically;
confirm biochemically.
–
Do biochemical
tests.
If not readily identifiable,
proceed to biochemical tests.

Figure 3. Isolation and preliminary identification.

TABLE 2. DIFFERENTIATION OF ENTEROBACTERIACEAE BY BIOCHEMICAL TESTS.

TEST or SUBSTRATE	ESCHERICHIEAE		EDWARDSIELLEAE	SALMONELLEAE			KLEBSIELLEAE									PROTEEAE					
								Enterobacter							*Pectobacterium*	*Proteus*				*Providencia*	
	Escherichia	*Shigella*	*Edwardsiella*	*Salmonella*	*Arizona*	*Citrobacter*	*Klebsiella*	*cloacae*	*aerogenes*	*hafniae*		*liquefaciens*		*Serratia*		*vulgaris*	*mirabilis*	*morganii*	*rettgeri*	*alcalifaciens*	*stuartii*
										37C	22C	37C	22C		22C						
INDOL	+	- or +	+	-	-	-	- or +	-	-	-	-	-	-	-	- or +	+	-	+	+	+	+
METHYL RED	+	+	+	+	+	+	-	-	-	+ or -	-	+ or -	- or +	- or +	+ or -	+	+	+	+	+	+
VOGES – PROSKAUER	-	-	-	-	-	-	+	+	+	+ or -	+	- or +	+ or -	+	- or +	-	- or +	-	-	-	-
SIMMONS'S CITRATE	-	-	-	d	+	+	+	+	+	(+) or -	d	+	+	+	d	d	+ or (+)	-	+	+	+
HYDROGEN SULFIDE (TSI)	-	-	+	+	+	+ or -	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
UREASE	-	-	-	-	-	d^{w}	+	+ or -	-	-	-	d	-	d^{w}	d^{w}	+	+	+	+	-	-
KCN	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+ or -	+	+	+	+	+	+
MOTILITY	+ or -	-	+	+	+	+	-	+	+	+	+	d	+	+	+ or -	+	+	+	+	+	+
GELATIN (22C)	-	-	-	-	(+)	-	-	(+) or -	- or (+)		-		+	+	+ or (+)	+ or (+)	+	-	-	-	-
LYSINE DECARBOXYLASE	d	-	+	+	+	-	+	-	+	+	+	+ or -	+	+	-	-	-	-	-	-	-
ARGININE DIHYDROLASE	d	- or (+)	-	(+) or +	+ or (+)	d	-	+	-	-	-	-	-	-	- or +	-	-	-	-	-	-
ORNITHINE DECARBOXYLASE	d	d(1)	+	+	+	d	-	+	+	+	+	+	+	+	-	-	+	+	-	-	-
PHENYLALANINE DEAMINASE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
MALONATE	-	-	-	-	+	d	+	+ or -	+ or -	+ or -	+ or -	-	-	-	- or +	-	-	-	-	-	-
GAS FROM GLUCOSE	+	-(1)	+	+	+	+	+	+	+	+	+	+	+	+ or -(3)	- or +	+ or -	+	d	- or +	+ or -	-
LACTOSE	+	-(1)	-	-	d	d	+	+	+	- or (+)	- or (+)	d	(+)	- or (+)	d	-	-	-	-	-	-
SUCROSE	d	-(1)	-	-	-	d	+	+	+	d	d	+	+	+	+	+	d	-	d	d	d
MANNITOL	+	+ or -	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+ or -	-	d
DULCITOL	d	d	-	d(2)	-	d	- or +	- or +	-	-	-	-	-	-	-	-	-	-	-	-	-
SALICIN	d	-	-	-	-	d	+	+ or (+)	+	d	d	+	+	+	+	d	d	-	d	-	-
ADONITOL	-	-	-	-	-	-	+ or -	- or +	+	-	-	d	d	d	-	-	-	-	d	+	-
INOSITOL	-	-	-	d	-	-	+	d	+	-	-	+	+	d	-	-	-	-	+	-	+
SORBITOL	+	d	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	d	-	d
ARABINOSE	+	d	-	+(2)	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
RAFFINOSE	d	d	-	-	-	d	+	+	+	-	-	+	+	-	+ or (+)	-	-	-	-	-	-
RHAMNOSE	d	d	-	+	+	+	+	+	+	+	+	-	-	-	d	-	-	-	+ or -	-	-

(1) Certain biotypes of *S. flexneri* produce gas; *S. sonnei* cultures ferment lactose and sucrose slowly and decarboxylate ornithine.
(2) *S. typhi*, *S. cholerae-suis*, *S. enteritidis* bioser. Paratyphi A and Pullorum, and a few others ordinarily do not ferment dulcitol promptly. *S. cholerae-suis* does not ferment arabinose.
(3) Gas volumes produced by cultures of *Serratia*, *Proteus*, and *Providencia* are small.

+, 90 percent or more positive in 1 or 2 days. –, 90 percent or more negative. d, different biochemical types [+, (+), –]. (+), delayed positive. + or –, majority of cultures positive. – or +, majority negative. w, weakly positive reaction.

*Source: *Identification of Enterobacteriaceae* by P.R. Edwards and W.H. Ewing. Third edition, 1972, p. 24. Reproduced by permission, Burgess Publishing Company, Minneapolis, Minnesota.

Method for Examination of Stack Effluents

As described in Methods for Collection of Incinerator Stack Effluents (using the Armstrong sampler), the microorganisms are impinged into a 300-ml phosphate buffer solution.

1. Filter 100 ml of the "inoculated" phosphate buffer solution through a 0.45μ HA membrane filter (3).
2. Transfer membrane filter with sterile forceps to a culture plate containing trypticase soy agar.
3. Incubate culture plate under constant saturated humidity for 20 hr (± 2 hr) at 35 C.
4. After incubation, remove cover from culture plate and determine colony count with the aid of a low-power (10-15 magnifications) binocular, wide-field microscope. Characterize colonies using specific isolation media.
5. Remove a 10-ml portion of the "inoculated" phosphate buffer solution and examine for viable heat-resistant spores as directed in steps 1 through 6 of the procedure under Method to Determine the Presence of Viable Heat-Resistant Spore Numbers.

Microbial counts are reported as organisms per cubic foot of air. If the sample is not taken under isokinetic conditions, the results are qualitative. If the stack velocity is known and remains relatively constant, however, the flow rate of the sampler can be adjusted to isokinetic conditions to yield quantitative results.

Method for Examination of Dust

As described in Methods for Collection of Dust Samples, the Andersen sampler is used with two types of media–trypticase soy agar (TSA-BBL product) containing 5 percent sheep blood, and eosin methylene blue agar (EMB-Difco product). The TSA/blood agar is used to isolate a wider range of fastidious organisms such as *Staphylococci*, *Streptococci*, and *Diplococci*. The EMB agar is used to isolate gram-negative bacteria. The plates are incubated aerobically at 37 C for 24 hr. (Preliminary studies showed that few organisms in the dust would grow under anaerobic conditions.) Enumeration of colonies is made with a Quebec colony counter. Microbial count is reported as organisms per cubic foot of air. At times, when microbial counts are high, the sampling time is 0.25 min, thus yielding 0.25 cu ft air.

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